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PTO/SB/05 (2/98)

JC490 U.S. PTO 06/03/99

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	LUD 5246.4 JEL/NDH
First Inventor or Application Identifier	Hiles, et al.
Title	METHOD FOR DETERMINING EXPRESSION OF A P13 KINASE GENE
Express Mail Label No.	EM004581915US

A

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ *Fee Transmittal Form (*e.g., PTO/SB/17*)
   (Submit an original and a duplicate for fee processing)
2. ☒ Specification *(preferred arrangement set forth below)* — *Total Pages* 62
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Reference of Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. ☒ Drawing(s) *(35 U.S.C. 113)* — *Total Sheets* 80
4. ☒ Oath or Declaration — *Total Pages* 4
   a. ☐ Newly executed (original or copy)
   b. ☒ Copy from a prior application (37 C.F.R. § 1.63(d))
   (for continuation/divisional with Box 17 completed)
      i. ☐ DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1 63(d)(2) and 1.33 (b)
5. ☒ Incorporation By Reference (*useable if Box 4b is checked*)
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be a part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

6. ☐ Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Copy
   b. ☒ Paper Copy (identical to computer copy)
   c. ☐ Statement verifying identity of above copies

jc549 U.S. PTO 09/325095 06/03/99

ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* ☒ Power of Attorney
10. ☐ English Translation Document *(if applicable)*
11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. ☐ *Small Entity Statement(s) (PTO/SB/09-12) ☒ Statement filed in prior application, Status is proper and desired
15. ☐ Certified Copy of Priority Document(s)
16. ☐ Other:

NOTE FOR ITEMS 1 & 14: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STSTEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28)

17. **If a CONTINUING APPLICATION,** ***check appropriate box, and supply the requisite information below and in a preliminary amendment:***

☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP) of prior application No: 09/085,957

Prior application information: Examiner: J. HINES Group / Art Unit: 1641

18. CORRESPONDENCE ADDRESS

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FULBRIGHT & JAWORSKI L.L.P.

By: Denise Squires
Denise Squires

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Hiles, et al.
Serial No.	:	Divisional of 09/085,957
Filed	:	Herewith
For	:	Method For Determining Expression of a PI3 Kinase Gene
Group Art Unit	:	Not Yet Assigned
Examiner	:	Not Yet Assigned

June 3, 1999

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend this application as follows:

IN THE TITLE

Replace the current title with

–Method For Determining Expression of a PI3 Kinase Gene--

IN THE SPECIFICATION

Page 1, after the title, add -- RELATED APPLICATIONS

This application is a divisional of Serial No. 08/085,957, filed May 27, 1998, which is a divisional of Serial No. 08/780,872, filed January 9, 1997, now U.S. Patent No. 5,846,824, which

is a divisional of Serial No. 08/162,081, filed February 7, 1994, now U.S. Patent No. 5,824,492, which is a national filing under 35 USC § 371 of PCT/GB93/21328.--

IN THE CLAIMS

Cancel claims 1-26 without prejudice, and add claims 27-36 which follow:

27. A method for determining expression of a gene which encodes a human polypeptide that has PI3 kinase activity and a molecular weight of about 110 kiladaltons as determined by SDS-PAGE, comprising contacting a sample with a nucleic acid molecule which hybridizes specifically to a transcript of said gene, and determining said hybridization as a determination of expression of said gene.

28. The method of claim 27, wherein said nucleic acid molecule is labelled with ^{32}P.

29. The method of claim 27, wherein said nucleic acid molecule is an antisense, RNA molecule.

30. The method of claim 27, wherein said nucleic acid molecule is a DNA molecule.

31. The method of claim 27, wherein said method comprises polymerase chain reaction.

32. The method of claim 27, wherein said nucleic acid molecule comprises a nucleotide sequence set forth in SEQ ID NO: 12, 14, 15, 16, 17, 18, 21, 22, 24, 25, 27 or 29.

33. The method of claim 31, comprising contacting said sample with a pair of oligonucleotide primers, said pair selected from the group consisting of (i) SEQ ID NOS: 12 and 14, (ii) SEQ ID NOS: 15 and 16, (iii) SEQ ID NOS: 17 and 18, (iv) SEQ ID NOS: 21 and 22, (v) SEQ ID NOS: 24 and 25, and (vi) SEQ ID NOS: 27 and 29.

34. The method of claim 27, wherein said sample is RNA isolated from a cell sample.

35. A method for determining if a cell contains a gene which encodes a human polypeptide which has PI3 kinase activity and a molecular weight of about 110 kilodaltons as determined by SDS-PAGE, comprising isolating DNA from said cell and contacting isolated DNA with a labelled nucleic acid molecule which hypridizes specifically to said gene and determining hybridization as a determination of presence of said gene.

36. A method for determining if a substance is an agonist or antagonist of expression of a gene which encodes a human polypeptide which has PI3 kinase activity and a molecular weight of about 110 kilodaltons as determined by SDS-PAGE, comprising contacting a sample which is known to contain said gene with said substance followed by contacting said sample with a nucleic acid molecule which hybridizes specifically to a transcript of said gene, determining hybridization to said transcript, and comparing hybridization to said transcript to hybridization of said nucleic acid molecule to said transcript prior to contact with said substance, differences therebetween indicating that said substance is an agonist or antagonist of expression of said gene.

REMARKS

Entry of the foregoing amendment is requested.

Respectfully submitted,

FULBRIGHT & JAWORSKI L.L.P.

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Polypeptides Having Kinase Activity, Their Preparation and Use

This invention relates to new polypeptides which exhibit kinase activity. More specifically, the invention is concerned with polypeptides which show phosphoinositide (hereinafter "PI") 3-kinase activity, particularly molecules involved in pathways responsible for cellular growth and differentiation.

Major advances have taken place in our knowledge of the structure and function of the signal transducing molecules and second messenger systems coupled to cell surface receptors. Thus, a subset of polypeptide growth factor receptors belong to the family of protein-tyrosine kinases (hereinafter "PTK" and activation of these receptors following ligand binding involves autophosphorylation of the receptor as well as phosphorylation of a number of intracellular substrate proteins (reviewed in Ullrich, A et al., 1990). The importance of receptor autophosphorylation had been unclear until recently, when evidence from several laboratories has suggested that this event may mediate the formation of complexes between receptor proteins and putative growth regulatory proteins such as phospholipase Cγ(PLCγ) (Meisenhelder et al, 1989), phosphatidylinositol PI3-kinase (Coughlin, S R et al, 1989). GTPase-activating protein (GAP) (Kaplan et al, 1990), the serine/theonine kinase Raf (Morrison et al, 1989), and members of the *src*-family of protein-tyrosine kinases (Kypta, R M et al., 1990) (reviewed in Cantley, L C et al., 1991).

The association of PI kinase activity with activated receptors is of particular interest since increased turnover of PI and its phosphorylated derivatives has been implicated in the action of hormones, growth factors and transformation of cells by DNA and RNA viruses

(reviewed in Whitman, M et al., 1988; Cantley et al., 1991). Several species of PI kinase are known to exist, but up to now none of these enzymes have been characterised by cloning and expression and the demonstration of PI kinase activity. Fibroblasts contain at least two PI kinase activities which are distinguishable on the basis of their detergent sensitivity and kinetic properties (Whitman, M et al., 1987). These two activities were classified as Type I (inhibited by non-ionic detergents) and Type II (stimulated by non-ionic detergents and inhibited by adenosine). A third distinct species (Type III) has been identified in bovine brain but remains poorly characterised (Enderman, G et al., 1987). One species of PI kinase activity in particular has become of major interest in the search for second messenger systems linked to protein-tyrosine kinases because this activity was shown to co-immunoprecipitate with activated platelet-derived growth factor (PDGF) receptors (Kaplan, D R et al., 1987; Coughlin, S R et al., 1989) and with the polyoma middle T antigen/pp60^{c-src} (mT:pp60^{c-src}) complex (Whitman, M et al., 1985). This activity has been shown to be due to a Type I PI kinase which produces novel inositol lipids phosphorylated at the D-3 position of the inositol ring (Whitman, M et al., 1988). More recently this enzyme has also been shown to associate with the CSF-1 receptor (Varticovski, L et al., 1989) kit (Lev et al, 1991), the epidermal growth factor (EGF) receptor (Bjorge et al, 1990), the PDGF α-receptor (Yu et al, 1991), the insulin receptor (Ruderman et al, 1990), the hepatocyte growth factor receptor, Met (Graziani et al, 1991), and with activated non-receptor protein-tyrosine kinases (Fukui & Hanafusa, 1989; Chan et al, 1990; Varticovski et al, 1991).

PI3 kinase activity has been closely linked to the presence of 81/85 kD proteins in these immunoprecipitates which can be phosphorylated on tyrosine residues by the

associated protein-tyrosine kinase both *in vitro* and in vivo (Kaplan, D R et al., 1987; Courtneidge, S A et al., 1987; Cohen et al, 1990). Recently a 650 fold purification of PI3-kinase from bovine brain was described which, among other proteins present in the purest preparation, contained an 85 kD protein which was shown to be an *in vitro* substrate for the PDGF and EGF receptors (Morgan, S J et al., 1990). Using sequence information from tryptic peptides derived from this protein, two homologous bovine p85 proteins, denoted p85α and p85β (Otsu, M et al., 1991) have recently been cloned. Two other groups have independently cloned murine and human p85α homologues using different strategies (Escobedo, J A et al., 1991b; Skolnik, E Y et al., 1991). Both of these p85 proteins can be demonstrated to bind directly to phosphorylated PDGF receptor *in vitro* (Otsu, M et al., 1991; Escobedo, J A et al., 1991b). These proteins may function as the receptor binding subunits of the PI3-kinase since neither of them can be shown to encode intrinsic PI3-kinase activity when expressed in a variety of cell systems (Otsu, M et al., 1991; Escobedo, J A et al., 1991b). However, immunoprecipitation of ^{125}I-labelled bovine brain PI3-kinase with antibodies raised against p85 proteins precipitates an 85 kD protein together with a second protein of molecular weight 110 kD (Otsu, M et al., 1991).

PI3-kinase is one of a growing number of potential signalling proteins which associate with protein-tyrosine kinases activated either by ligand stimulation or as a consequence of cell transformation. A common feature of all these proteins (apart from Raf), is that they contain one or more SH2 domains (*src* homology) (Koch, C A et al., 1991). Both p85α and p85β proteins contain two SH2 domains. Experiments from a number of laboratories have suggested that these domains may function by binding to

peptide sequences usually phosphorylated on tyrosine residues, and thus mediate the complex formation which follows activation of protein-tyrosine kinases (Anderson et al, 1990; Meyer & Hanafusa, 1990; Moran et al, 1990; Matsuda et al, 1991; Meyer et al, 1991; reviewed in Koch, C A et al., 1991). In support of this, several studies suggest that tyrosine phosphorylation of the PDGF receptor or polyoma mT is essential for its association with proteins such as the PI3-kinase (Kazlauskas, A et al., 1989; Talmage, D A et al., 1989) GAP (Kaplan et al, 1990; Kazlauskas, A et al., 1990) and PLCγ (Anderson et al, 1990; Margolis et al, 1990). The precise tyrosine residue required for binding of the PI3-kinase activity (and an 85 kD phosphoprotein) to the human PDGF receptor has been mapped to tyrosine 751 which lies within the kinase insert region of the protein-tyrosine kinase domain (Kazlauskas & Cooper, 1989, 1990; Kazlauskas et al, 1991). The binding sites for other proteins to this receptor (eg., PLCγ, GAP and *src*-family kinases) have yet to be mapped, but these proteins may associate via other phosphorylated tyrosine residues.

This invention has been facilitated by the finding that certain synthesized peptides from the human PDGF β-receptor, namely peptides derived from the sequence around tyrosine 751 of the PDGF receptor, can be used to bind and isolate bovine brain PI3-kinase, making it possible to purify further partially purified bovine brain PI3-kinase (as described by Morgan et al, 1990) to apparent homogeneity and to obtain reasonably pure p110 protein. As will be described hereinafter, the PI3-kinase requires a phosphopeptide column containing a YXXM motif for its isolation by such a technique, the tyrosine being phosphorylated. Only if a column of this type is used are both the 85 kD and 110 kD proteins secured whereas 85 kD subunit binds to all phosphopeptide affinity columns tested and only fails to bind to non-

phosphorylated peptides. Moreover, the relatively small size of the phosphopeptides used for such columns gives good specificity and a high density of affinity groups per unit volume of column.

This purification has allowed amino acid sequence information to be provided, and cDNA cloning to be performed. Such cloning has revealed some interesting facts. Thus, p110 is a 1068 amino acid protein having an unexpectedly high (compared to SDS-PAGE Figures) calculated molecular weight of about 124 kD (124247). The protein is related to Vps34p, a Saccharomyces cerevisiae protein involved in the sorting of proteins to the vacuole. Surprisingly, p110 when expressed in COS-1 cells was inactive and activity was only seen when complexed with p85. However, when expressed in insect cells, p110 could be shown to possess intrinsic kinase activity. The novel p110 polypeptide can be associated with p85α into an active p85α/p110 complex which binds the activated colony stimulating factor-1 receptor. The invention is also based upon these discoveries and unpredictable findings.

Thus, in one aspect the present invention provides an isolated polypeptide of calculated molecular weight approximately 124 kD which possesses PI3-kinase activity when produced by recombinant production in insect cells, or a polypeptide derivable therefrom which has PI3-kinase activity and binds, when associated with a p85 mammalian PI3 kinase subunit, to a phosphopeptide which includes the YXXM motif, the tyrosine being phosphorylated. Such polypeptides are preferably those capable of association with p85 subunits of mammalian PI3-kinases to produce active p85/p110 complexes. Preferably, the polypeptides have either the amino acid sequence of Figure 9 hereof or exhibit significant sequence homology therewith. Preferred are polypeptides having at least amino acids 272 to 1068 of the seqeunce of Figure 9 hereof.

As used herein, the term "PI3-kinase activity" means phosphoinositide-3 kinase activity.

The invention embraces polypeptides as defined and exhibiting sequence homology with any chosen mammalian species of PI3-kinase. A human sequence is given in Figure 16 hereof. Amino acids 37(tyr)-834 (stop codon) (see Figure 16) are >99% conserved with the bovine p110 cDNA sequence and correspond to amino acids 272-1069 (stop codon) of the sequence of Figure 9. Upstream of amino acid 37 (human sequence) there is no sequence similarity between the p110 cDNA sequences from the two species.

The invention includes antibodies, monoclonal or otherwise, against the polypeptides of the invention.

In another aspect the invention includes a DNA sequence comprising either: (a) a sequence set out in Figure 9 hereof; (b) any one of the subsequences A to N of Figure 9 hereof; (c) the sequence represented by bases 816 to 3204 of Figure 9 hereof; (d) a sequence set out in Figure 16 hereof; or (e) a DNA sequence hybridizable to (a), (b), (c) or (d); which sequence (a), (b), (c), (d) or (e) encodes a polypeptide which has PI3-kinase activity if expressed in insect cells or can complex with a p85 mammalian PI3-kinase subunit to produce such activity. Subsequences A to N, referred to above, are themselves part of the present invention.

Hybridization conditions which may be used to find active sequences include, but are not limited to, 1 M NaCl/10 x Denhardt's solution/50 mM Tri-HCl (pH 7.4)/ 10 mM EDTA/ 0.1% SDS/100 μg/ml denatured herring sperm DNA (Sigma) at 65°C for 16 h, with the following washing conditions, i.e. 2 x SSC/0.1% SDS, 42°C---->0.5 x SSC/0.1% SDS, 50°C---->0.1 x SSC/0.1% SDS, 65°C---->0.1 x SSC/0.1% SDS, 68°C.

The invention further provides a DNA construct comprising a DNA sequence as defined above under the control of a control sequence and in proper reading frame in an expression vector.

The control sequence may include a regulatable promoter (e.g. Trp). Selected host cells which have been genetically altered to permit expression of the encoded polypeptide by the incorporation of such a construct are another aspect of the invention, and the invention also includes both a method of making such a polypeptide by cultivating such host cells and, of course, the resulting polypeptides.

In general, new polypeptides of the invention can be used to provide PI3-kinase activity, either directly or after complexing with a mammalian p85 subunit. Enzymatically active complexes involving the above-defined polypeptides are part of the invention.

The invention envisages a method of prophylaxis or therapy which involves the encouragement or discouragement of cell proliferation by the action of an agonist or antagonist, respectively, for the PI3-kinase activity of a polypeptide of the invention or complex including the same, wherein said cell proliferation is mediated through a cell surface receptor interactive with said activity. The present invention opens up for the first time, by providing pure sequenced active protein, the opportunity to screen (using standard techniques) for such agonists or antagonists.

Another aspect of the invention is a pharmaceutical or veterinary formulation comprising an agonist or antagonist as defined above formulated for pharmaceutical or veterinary use, respectively, optionally together with an acceptable diluent, carrier or excipient and/or in unit dosage form. Conventional pharmaceutical or

veterinary practice may be employed to provide suitable formulations or compositions.

Thus, the formulations of this invention can be applied to parenteral administration, for example, intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperiotoneal, topical, intranasal, aerosol, scarification, and also oral, buccal, rectal or vaginal administration.

Parenteral formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are to be found in, for example, "Remington's Pharmaceutical Sciences". Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymers, lactide/glycolide copolymers, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present factors. Other potentially useful parenteral delievery systems for the factors include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, lactose or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also

include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The concentration of PI3-kinase agonist or antagonist in the formulations of the invention will vary depending upon a number of issues, including the dosage to be administered, and the route of administration.

In general terms, such agonists or antagonists may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. General dose ranges are from about 1 μg/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.01 mg/kg to 100 mg/kg of body weight per day. The preferred dosage to be administered is likely to depend upon the type and extent of progression of the condition being addressed, the overall health of the patient, the make up of the formulation, and the route of administration.

The invention also includes the use of a polypeptide of the invention, or active complex containing the same, or an agonist or antagonist thereof in affecting the level of stimulation of platelets or neutrophils or in regulating blood glucose levels (the action of insulin may be mediated by PI3-kinase activity), and such use when employed for prophylactic or therapeutic purposes is envisaged.

The polypeptides of the invention (or complexes containing them) have a particular utility in the in vitro enzymatic production of 3-phosphorylated phosphoinositides eg PI(3)P, PI(3,4)P2, PI(3,4,5)P3). Such materials are of considerable biochemical interest, and are often very difficult to synthesize by conventional chemical techniques. This invention provides, for the first time, appreciable amounts of

purified and reliable enzymatic activity for such in vitro synthesis.

In general, the first step in the purification and cloning upon which the invention is based involved partial purification of PI3-kinase from bovine brain as previously described (Morgan et al, 1990) and then further purification by affinity chromatography on an immobilised 17 amino acid phosphotyrosine peptide whose sequence is based on that surrounding tyrosine 751 of the human PDGF-β receptor. Following this final purification, p110 and p85 were eluted from the resin with SDS-containing buffers. The p85/p110 mixture was either digested directly with lysylendopeptidase, or p110 was further purified by SDS-agarose gel electrophoresis (see below) and digested following elution from the gel. Peptides were separated by reverse phase HPLC and sequenced using a modified Applied Biosystems 477A sequencer. Amino acid sequence analysis of 14 peptides (A to N, Figure 9) generated 235 residues which could be assigned with certainty (see Figure 9, attached).

It is important to note that the successful production of sequence information herein was dependent upon a novel SDS-agarose gel electrophoresis technique. Although, SDS-PAGE is widely used for high resolution protein separations, and is a method which resolves components primarily by their differences in molecular weight, as the polyacrylamide matrix is not readily disrupted, protein recovery following SDS-PAGE generally requires techniques involving electroelution from gel slices, electroblotting, or passive diffusion. Elution of proteins from polyacrylamide gels that have been previously stained using sensitive reagents (such as Coomassie Blue) is slow and recoveries are frequently low. Furthermore, these methods may concentrate impurities present in the polyacrylamide matrix and in the relatively large buffer volumes required for elution.

Preparative SDS-PAGE systems using continuous flow collection have also been developed, but these frequently exhibit decreased resolution and low recoveries.

The novel method employed herein uses SDS-agarose gel electrophoresis (SDS-AGE) and allows a combination of the high resolving capacity of slab gel electrophoresis and

the detection of proteins using sensitive stains with a rapid recovery technique that isolates proteins in high yield and in small volumes. The recovered protein is highly purified and in a form that can be either readily precipitated or digested directly in SDS containing buffers. Peptides produced by this method can be fractionated by HPLC and then analysed by automated amino acid sequencing. The recovery of long hydrophobic peptides is particularly efficient using these digestion conditions. The following protocol guides the skilled reader.

PROTOCOL

Materials

All chemicals should be of analytical or purer grades. Guanidinium hydrochloride was Aristar grade (BDH, UK). FMC Prosieve was purchased from Flowgen (UK) and ultrapure agarose was from BRL (USA). Other electrophoresis reagents were from Biorad (UK, Electrophoresis grade). Standard molecular weight proteins were from Bio-Rad (UK) and Amersham International (UK). Sequencing grade trypsin (porcine, EC 3.4.21.4) was from Boehringer Mannheim (UK) and lysylendopeptidase (Achromobacter lyticus, EC 3.4.21.50) was from Wako Chemicals GmbH (Germany). Glass capillaries were those supplied by Applied Biosystems Inc (USA) for use on the 430A HPEC system, but were frosted by abrasion with an aqueous carborundum suspension (C150 grade) and a steel rod. Frosted slab gel plates were

obtained from Hoefer (UK).

Slab SDS-AGE

Slab Prosieve resolving gels of 0.75 or 1.5 mm thickness were poured essentially as described by the manufacturer using pairs of 16 x 18 cm glass plates, one of which was frosted in order to prevent the gel from slipping out of the electrophoresis assembly. It is important to ensure that the gel plates be thoroughly warmed to 60°C prior to pouring the resolving gel. The inability to warm the gel plates prior to pouring an agarose stacking gel, the insertion of the comb into a rapidly cooling gel, and the removal of the comb from the fragile agarose stacking gel initially caused severe problems. In order to remove these difficulties a 5%T, 2.6%C polyacrylamide stacking gel was used in place of agarose in later preparations.

Samples were denatured at 100°C in sample buffer (190 mM Tris/HCl, pH 6.8, 6% (w/v) SDS, 30% (v/v) glycerol, 10 mM DTT, 0.01% (w/v) bromophenol blue) and gels were run using Laemmli cathode buffer (0.192 M glycine, 0.025 M Tris, 0.1% (w/v) SDS) with a modified anode buffer (1M Tris/HCl, pH 8.3) at 200 v (approximately 50 mA for 1.5 mm and 25 mA for 0.75 mm gels) for about 4h using a SE400 gel apparatus (Hoefer, USA). Gels were stained using either Coomassie Blue G-250 (Bio-Rad, UK) with rapid destaining or 4M ammonium acetate solution. In the latter case proteins were identified within a few minutes by optical contrast using incident light reflection observed against a dark background. Protein bands were immediately excised and gel slices stored at -20°C.

HPEC Electroelution

Gel slices were thawed and washed twice in 1 ml of 62.5 mM Tris/HCl, pH 6.8 for 5 min each at 20°C. Slices containing Coomassie Blue were prewashed with 1 ml of 50% (v/v) methanol, 5% (v/v) acetic acid for 5 min at 20°C.

The volume of the gel slice was estimated, then 10% SDS and 20% DTT were added to final concentrations of 2% and 0.2% (w/v) respectively. The gel slice was melted and homogenized by immersion in boiling water for 5 min with occasional mixing. The sample volume was then measured and made up to the required amount (see Table 1 below) with prewarmed 62.5 mM Tris/HCl, pH 6.8. The diluted

sample was heated for a further 5 min and loaded into a prewarmed glass HPEC capillary. It was important not to exceed 90% of the capillary volume at this stage. The capillary was incubated at 4°C for at least 10 min to allow the sample gel to solidify, before the slow addition of 0.8% agarose, 1 M Tris/HCl, pH 8.8 to overfill the capillary. After a further 10 min at 4°C, the ends of the gel were trimmed flush, sealed with Zytex discs, and applied to an Applied Biosystems 230A HPEC system. Electroelution was performed using an elution buffer pressure of 2.5 psi (generating a flow rate of approximately 1 μl/min), an upper reservoir buffer pressure of 3.5 psi and a lower reservoir buffer pressure of 0.9 psi. These settings were changed from the manufacturer's recommendations in order to stop the gel from collapsing upwards during the run. The current settings were as described in the text and 3 min fractions were collected while monitoring the eluate at 280 nm. The fraction collector rack was cooled to 4°C and the gel compartment was cooled to 10°C.

Table 1

HPEC Elution Gel Parameters

Capillary size (mm)		**Gel volume (μl)[a]**			**Current (mA)**
Length	**i.d.[a]**	**Total**	**Sample**	**Focussing**	
50	2.5	245	220	25	1,0-1.5
50	3.5	480	432	48	1.5
100	2.5	491	441	49	2.0-2.5
100	3.5	960	864	96	2.5

[a] These values are underestimated due to the variable increase in the internal diameter of the capillaries caused by the frosting procedure.

Preparation of Proteins for Sequence Analysis

Fractions were assayed for protein content and purity either by monitoring radioactivity or by SDS-PAGE and silver staining. Samples required for trypsin or lysylendopeptidase digestion and subsequence sequence analysis were separated from Coomassie Blue by sequential precipitation on ice using 10% (w/v) TCA and then 20% TCA with centrifugation for 10 min at 4°C. Pellets were washed with 1 ml of acetone at -20°C overnight and then washed again briefly in order to remove trace contamination by TCA and SDS before air drying and the addition of the required digestion buffer. Tryptic digestions were performed in 0.1 M Tris/HCl, pH 8.0 at 37°C and lyslendopeptidase digestions in 20 mM Tris/HCl, pH 8.8 containing 0.1% (w/v) SDS at 30°C. Solid guanidinium hydrochloride was added to tryptic digests (6M final concentration) and incubated for 1 h at 37°C. Products were applied directly to HPLC columns using a Hewlett-Packard 1090M system and the effluent was monitored with a 79880A diode array detector. Trypsin digests were fractionated using an Applied Biosystems RP-300 column (2.1 x 100 mm) while lysylendopeptidase products required an Applied Biosystems AX-300 (2.1 x 30 mm) and an OD-300 column (2.1 x 100 mm) connected in series essentially as described by Kawasaki and Suzuki (1990).

The following Examples are given to illustrate the present invention without limiting the same. The Examples refer to the accompanying drawings.

In the accompanying drawings:-

Figures 1 to 9 are concerned with Example 1, sections A and B.

Figure 1. Phosphorylation and purification of Y751 phosphopeptide.

Panel A. HPLC profile for separation of the phosphorylated from the non-phosphorylated Y751 peptide on a C_{18} reverse phase column. The trace shows the spectra monitored at 214 nm during the course of the elution. The peaks corresponding to the phosphorylated and non-phosphorylated peptide are indicated by arrows. The small peaks observed are derived from the A431 membranes.

Panel B. Spectral analysis of the purified phosphorylated and non-phosphorylated Y751 peptides between 240 and 300 nm as measured by the diode-array detector. The absorption maximum for the peptide is observed to shift to a lower wavelength following tyrosine phosphorylation.

Panel C. Phosphoamino acid analysis of Y751 peptide phosphorylated by either purified EGF receptor (left panel) or A431 cell membranes (right panel). Following the phosphorylation reaction the phosphopeptide was purified by reverse phase HPLC. The peptide was subjected to acid hydrolysis and the phosphoamino acids separated by two-dimensional thin layer electrophoresis. Internal standards were stained with ninhydrin and the ^{32}P-labelled phosphoamino acids were detected by autoradiography. The positions of inorganic phosphate (P_i), and phosphoserine (S), phosphothreonine (T) and phosphotyrosine (Y) standards are indicated.

Figure 2. Purification of PI 3-kinase complex on the Y751 phosphopeptide affinity column.
Panel A. Peak 1 (P1) and peak 2 (P2) of PI 3-kinase

fractions from the second MonoQ step were analysed on a 7.5% SDS-PAGE gel. Proteins in these two peak fractions were visualised by silver staining. The migration positions of molecular weight markers are indicated.

Panel B. Affinity purification of peak 1 (P1) and peak 2 (P2) PI 3-kinase using the Y751 phosphopeptide column. Silver stain of a 7.5% SDS-PAGE gel showing PI 3-kinase associated proteins from MonoQ P1 and P2 which bound to, and were eluted from, the Y751 phosphopeptide column with 0.1% SDS-containing phosphate buffer at 80°C. Lanes 1, 2 and 3 for both the P1 and P2 material indicates the proteins eluted by successive 50 μl elutions.

Figure 3. Characterisation of the binding of PI 3-kinase activity to Y751 derived peptide columns.
One microgram of partially purified peak 1 bovine brain PI 3-kinase was applied to 10 μl of the Y751 derived peptide resins in 100 μl of binding buffer. Bound proteins were assayed for PI 3-kinase activity. Lane 1, PI 3-kinase activity bound to non-phosphorylated Y751 column. Lane 2, PI 3-kinase activity bound to phosphorylated Y751 column. Lane 3, PI 3-kinase activity removed from supernatant of column in lane 2 by fresh phosphorylated Y751 column. Lane 4, PI 3-kinase activity remaining associated with the column from lane 2 following removal of the bound material using 0.1% SDS at 80°C. Lane 5, PI 3-kinase activity bound to recycled phosphorylated Y751 column as used in lane 2 following addition of a fresh aliquot of bovine brain PI 3-kinase in binding buffer. Lane 6, Equivalent amount of peak 1 soluble bovine brain PI 3-kinase activity as applied to columns in lane 2 or lane 5.

Figure 4. Identify of p85 species in peak 1 and 2 of bovine brain PI 3-kinase preparation.
Protein samples were separated on 7.5% SDS-PAGE gels and

transferred to nitrocellulose. The blots were then probed with antisera raised against the COOH-terminal peptide sequences of p85α or p85β.

Panel A. Western blot probed with anti-p85α COOH-terminal antisera.

Lane 1, peak 1 bovine brain PI 3-kinase; lane 2, peak 2 bovine brain PI 3-kinase; lane 3, Cos-1 cell lysate from pMT2 vector alone transfected cells; lane 4, Cos-1 cell lysate from pMT2p85α transfected cells; lane 5, Cos-1 cell lysate from pMT2p85β transfected cells; lane 6 Sf9 cell lysate containing p85α; lane 7, Sf9 cell lysate containing p85β Panel B. Western blot probed with anti-p85β COOH-terminal antisera.

Lanes are as described for panel A.

Panel C. Competition of peptides with antibodies in Western blots. Samples in lanes 1 and 2 were probed with p85α specific antiserum while samples in lanes 3 and 4 were probed with the p85β specific antiserum. Lanes 1 and 2. Sf9 cell lysate containing baculovirus expressed p85α. Lanes 3 and 4, Sf9 cell lysate containing baculovirus expressed p85β. In the odd numbered lanes the nitrocellulose was probed with specific antiserum alone. In the even numbered lanes the antiserum was competed with 100 μg/ml of p85α (lane 2) and p85β (lane 4) specific C-terminal peptides respectively.

Panel D. Anti p85α western blot of bound and soluble PI 3-kinase material after chromatography using the Y751 phosphopeptide column.

Peak 1 (P1) and peak 2 (P2) of bovine brain PI 3-kinase were immobilised on the Y751 phosphopeptide column. Material which did not bind was collected and then the

resin was washed extensively. Bound proteins were eluted from the column with SDS-PAGE sample buffer. Bound and unbound proteins were separated by SDS-PAGE on a 7.5% gel and then transferred to nitrocellulose. The filter was then probed with anti-p85α COOH-terminal antisera and

visualised with ^{125}I Protein A-Sepharose. Lane 1, P1 bound material; Lane 2, peak 1 material which did not bind to column; Lane 3, peak 2 bound material; Lane 4, peak 2 material which did not bind to column.

Figure 5. Specificity of binding of PI 3-kinase complex to Y751 peptide column:-comparison with Y857 phosphopeptides.
Sf9 cell lysates containing p85α proteins or one microgram of partially purified bovine brain PI 3-kinase (P1 and P2 MonoQ) was allowed to bind to the columns for 4 h at 4°C as described. The columns were then washed repeatedly with binding buffer, bound proteins were eluted with SDS-containing buffers and then analysed by electrophoresis on 7.5% SDS-PAGE gels. Bound proteins were visualised by silver staining. Panel A. Proteins bound to Y751 phosphopeptide column. Panel B. Proteins bound to Y857 phosphopeptide column. The migration position of molecular weight markers are indicated.

Figure 6. Binding of recombinant baculovirus expressed p85 proteins to a panel of phosphopeptide columns.
P85 proteins in SF9 cell lysates were tested for their ability to bind to the various peptide column. After extensive washing, bound proteins were eluted from the columns, separated on 7.5% SDS-PAGE gels and the visualised by staining with Coomassie Blue. Panel A. Bound p85α. Panel B. Bound p85β. CON, 17 amino acid non-phosphorylated Y751 column; Y751, 17 amino acid phosphopeptide from the kinase insert region of the PDGF β-receptor; Y751.S, 11 amino acid version of Y751 phosphopeptide; Y857, 17 amino acid phosphopeptide

derived from the sequence around the second major tyrosine phosphorylation site in the PDGF β-receptor; pGAT, poly Glu:Ala:Tyr phosphopeptide; Y416 and Y527, 13 and 16 amino acid phosphopeptides derived respectively from the two major tyrosine phosphorylation sites of $pp60^{c-src}$.

Figure 7. The p85/100 complex and PI 3-kinase activity show specificity in the range of phosphopeptides to which they will bind.
One microgram of partially purified bovine brain PI 3-kinase (P1 MonoQ) was allowed to bind to peptide affinity columns for 4 h at 4°C as described. The columns were then washed repeatedly with binding buffer. Bound proteins were then either eluted with SDS-containing buffers and then analysed by electrophoresis on 7.5% SDS-PAGE gels or assayed for PI 3-kinase activity bound to the column.

Panel A. Bound proteins were visualised by silver staining. The migration of molecular weight markers is indicated.

Panel B. PI 3-kinase activity bound to various phosphopeptide columns. The ^{32}P-labelled lipid products were separated by TLC and the visualised by autoradiography. PI3P indicates the migration position of a P13P standard. Ori indicates the origin of the TLC plate.

Figure 8. Binding of PI 3-kinase activity of phosphopeptides containing the YXXM motif.
Panel A. One microgram of partially purified peak 1 bovine brain PI 3-kinase was bound to 10 μl of the indicated peptide columns. Following extensive washing the columns were assayed for bound PI 3-kinase activity. Lane 1, PI 3-kinase activity bound to non-phosphorylated Y751 column; Lane 2, PI 3-kinase activity bound to

phosphorylated Y751 column; Lane 3, PI 3-kinase activity bound to phosphorylated Y751.S column; Lane 4 PI 3-kinase activity bound to phosphorylated Y857 column; Lane 5, PI 3-kinase activity bound to phosphorylated Y740 column;

Lane 6, PI 3-kinase activity bound to phosphorylated Met Y1313 column. PIP indicates the migration position of a P14P standard. Ori indicates the origin of the TLC plate.

Panel B. Comparison of identified PI 3-kinase binding sites in the peptides tested. The proposed consensus sequence for binding is also shown for comparison (Cantley et al., 1991).

Figures 9 to 15 are concerned with Example 1, sections C and D, and Figures 16 to 25 relate to Example 2.

Figure 9. Nucleotide Sequence and Deduced Amino Acid Sequence of p110.
(Top Panel) The nucleotide sequence of the coding region and the deduced amino acid sequence in one letter code are shown. Peptide sequences (lettered from A-N) obtained by protein sequencing are highlighted.

(Lower Panel) Schematic representation of the p110 cDNA. The bold line indicates coding sequence. (p2.1): extent of clone p2.1, (Race Product): region amplified by RACE PCR, (a): probe used in Southern blot analysis, (b): probe used in northern blot analysis, (S): Sau3AI site changed to BamHI site for expression in Sf9 cells.

Figure 10. Comparison of p110 and Vps34p Protein Sequences
(A) Dot plot comparison of Vps34p (875 amino acids: horizontal axis) and p110 (1068 amino acids: vertical axis) using the Compare program (UWGCG package; Devereux et al., 1984).

(B) The optimal alignment of p110 (upper sequence) and Vps34p (lower sequence) over the region of homology, using the Gap program (UWGCG package: Devereux et al., 1984). Identical residues are indicated by (I), conserved residues are indicated by (:). Residues proposed to be involved in ATP binding are marked with (*).

Figure 11. Genomic Southern Analysis of p110
High molecular weight DNAs (3 μg) of bovine (lanes 1, 2, 3), human (lanes 4, 5, 6) and rat (lanes 7, 8, 9) origin were digested with EcoRI (lanes 1, 4, 7), BamHI (lanes 2, 5, 8) of Hindlll (lanes 3, 6, 9), fractionated through a 0.5% agarose gel and transferred to a nitrocellulose membrane as described in Example 1. The filter was probed with a ^{32}P-labelled Xbal-Pstl fragment (probe a in Figure 9, lower panel). The filter was washed in 0.5 x SSC, 0.1% SDS at 50°C and exposed overnight (Panel A). The filter was then washed in 0.1 x SSC, 0.1% SDS at 68°C and exposed for seven days (Panel B). The marker track shows the positions of lambda Hindlll markers.

Figure 12. Analysis of Tissue Distribution of p110 Message
(A) Northern Blot Analysis of p110
5 μg of poly(A)$^+$RNA isolated from total bovine brain (lane 1) or the SGBAF-1 cell line (lane 2) were fractionated on a 0.9% agarose gel and immobilised on membranes as described in Example 1. The filter was probed with a ^{32}P labelled antisense RNA probe (probe b in Figure 9, lower panel). After washing in 0.1 x SSC, 0.1% SDS at 60°C, the filter was treated with 1 μg ml^{-1} RNAase A and autoradiographed overnight.

(B) PCR Analysis to Detect p110 Transcripts
Poly(A)$^+$ RNA was isolated from various sources and PCR

performed as described in Example 1. Bands of 218 bp and 212 bp indicate the specific amplification of human and bovine transcripts, respectively. Lane 1; Human T-cell blasts, lane 2; Human peripheral blood acute lymphocytic leukaemia cells, lane 3; A431 cells (Human), lane 4; COS-1 cells (Simian), lane 5; bovine brain, lane 6; SGBAF-1 cells (Bovine), lane 7; ZNR cells (Porcine).

(C) PCR Analysis to Detect p85α Transcripts
Poly $(A)^+$ RNA was isolated from various sources and PCR performed. Specific amplification of p85α message gives a bind of 190 bp. Lanes are the same as indicated for (B).

Figure 13. Expression of p85α and p110 in Sf9 Cells Using Baculovirus Vectors
(A) Sf9 cells were infected with a wild type baculovirus (lanes 1 and 2) or with baculoviruses expressing p85α (lane 3), p110 (lane 4) or p85α and p110 (lanes 5 and 6). Immunoprecipitates were prepared with either anti-p85α (lanes 1, 3, and 5) or anti-p110 antisera (lanes 2, 4 and 6), samples fractionated on a 7.5% SDS-PAGE gel and visualised by staining with Coomassie blue.

(B) PI3-kinase assays were performed on Immmunoprecipitates of p85α and p110 expressed in Sf9 cells. lanes 1-6 the same as Panel (A); lane 7: pI3-kinase activity from 1 μl of the partially purified bovine brain PI3-kinase preparation.

Figure 14. In Vitro Association of PI3-Kinase Activity with the CSF-1 Receptor
An in vitro PI3-kinase assay was performed on anti-CSF-1 receptor immunocomplexes prepared from Sf9 cells infected with a baculovirus expressing the CSF-1 receptor and treated as follows; lane 1: anti-CSF-1 receptor immunoprecipitates, untreated; lane 2; anti-CSF receptor immunoprecipitate, pre-treated with ATP and incubated

with a p85α/p110 containing Sf9 cell lysate; lane 3: anti-CSF-1 receptor immunoprecipitate, treated in the absence of ATP and incubated with a p85α/p110 containing Sf9 cell lysate; lane 4: anti-CSF-1 receptor immunoprecipitate, pre-treated with ATP and incubated with a p85α containing Sf9 cell lysate; lane 5; anti-CSF-1 receptor immunoprecipitate, pre-treated with ATP and incubated with a p110 containing Sf9 cell lysate.

Figure 15. Expression of p85α and p110 in COS-1 Cells COS-1 cells were transfected with 5 μg of the respective DNAs and harvested 48 h later. Transfected cells were labelled with 100 μCi ml^{-1} of ^{35}S-methionine for the last 4 h of this period. Immunoprecipitations were performed with either an p85α polyclonal antiserum or a p110 C-terminal peptide antiserum. After washing, the pellet was divided in two and half was then analyses on a 10% SDS-PAGE gel while the other half was subjected to P13-kinase assay.

(A) ^{35}S-labelled proteins immunoprecipitated with anti-p85α antiserum.

(B) PI3-kinase activity immunoprecipitated with anti-p85α antiserum.

(C) ^{35}S-labelled proteins immunoprecipitated with 110 C-terminal peptide antiserum.

(D) p13-kinase activity immunoprecipitated with 110 C-terminal peptide antiserum.

Lanes contain results from COS-1 cells transfected with the following DNAs; lane 1: vector DNA, lane 2: pMT2-p85α, lane 3: pSG5-p110, lane 4: pMT2-p85α and pSG5-110, lane 5 in panels B and D show the PI3-kinase activity immunoprecipitated with the two antisera from 1 μl of the

partially purified bovine brain p13-kinase preparation. The exposure times for panels A and C, and B and D are identical.

Figure 16. cDNA for human p110
The figure shows the sequence of human p110 cDNA, together with the corresponding amino acid sequence.

Figure 17. A comparison of the human p110 sequence and bovine p110 sequence at the DNA level.

Figure 18. A comparison of the human p110 sequence and bovine p110 sequence at the protein level.

Figure 19. The protein sequence of human p110.

Figure 20. The sequence of a cDNA related to p110, PITR-c.

Figure 21. The sequence of a cDNA related to p110, PITR-f.

Figure 22. The alignment of human p110, PITR-c, PITR-f and the yeast PI3-kinase VPS34.

Figure 23A. SDS PAGE analysis of proteins able to bind to various domains of human p110.

Figure 23B. Schematic representation of the domains of p110 analysed for their ability to bind p85.

Figure 24. Various deletion mutants and PCR fragments of p110 fragment p110-N.

Figure 25. The ability of the various deletion mutants and PCR fragments of p110-N to bind the p85 subunits.

Example 1

PROTEIN PURIFICATION

A. Methods and Materials

Cells

A431 cells were maintained in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. Maintenance of insect cell culture and infection of Spodoptera frugiperda (Sf9) cells were carried out as described in Summers and Smith (1987).

Preparation of A431 Membranes

This preparation was modified from that described by Thom et al (1977). Harvesting solution (0.05 M boric acid (pH 7.2), 0.15 M NaCl), extraction solution (0.02 M boric acid (pH 10.2), 0.2 mM EDTA) and borate solution 0.5 M Boric acid (pH 10.2) were all prepared fresh. Cells were washed once with ice-cold harvesting solution and then scraped into fresh harvesting solution. Cells were pelleted by low speed centrifugation at 200g, and then resuspended by pipetting in 2 pellet volumes of harvesting solution. This was added slowly, with stirring, to 100 pellet volumes of extraction solution. After 10 min, 8 pellet volumes of borate solution was added and stirring continued for a further 5 min. This solution was filtered through nylon gauze (Av. mesh size 900 μm), and spun at 500g for 10 min at 2°C to pellet any nuclei/whole cells. Finally, the supernatant was centrifuged at 12,000g in a ultracentrifuge SW28 rotor at 4°C for 30 min. The membrane pellet was resuspended in a minimum volume of 50 mM Hepes (pH 7.5) and stored at -70°C.

Synthesis of Peptides

Peptides described in Table 2 below were synthesized on an Applied Biosystems 430A peptide synthesizer using FMOC

chemistry and an appropriate amino acid addition program according to ABI's recommendations. Peptides were then purified by preparative reverse-phase HPLC. Composition of the peptides was checked by analytical HPLC, amino acid analysis and protein sequencing on an 477A automated pulse-liquid sequencer.

Table 2

Peptide	**Sequence**
Y740	G E S D G G Y M D M S K
Y751	D M S K D E S V D Y V P M L D M K
Y751.S	C D E S V D Y V P M L
Y857	A R D I M R D S N Y I S K G S T F
Y1313	E F C P D P L Y E V M L K
Y527	R R F T S T E P Q Y Q P G E N L
Y416[a]	R R L I E D N E Y T A R G

[a] This peptide was purchased from Sigma Chemical Co Ltd rather than synthesized.

Phosphorylation of Peptides

Peptides were lyophilised to dryness to remove any contaminating chemicals remaining from synthesis/purification and then dissolved in HPLC grade water at a concentration of ~4 mg/ml.

For small scale phosphorylation: 20 μg of peptide, 10 μl 5x kinase buffer (250 mM Hepes (pH 7.4), 750 mM NaCl, 0.1% Triton X-100, 10 nM $MnCl_2$, 60 mM $MgCl_2$, 50% glycerol, 500 mM sodium orthovanadate), 5 μl A431 membrane preparation and ATP/[γ-^{32}P]ATP (relative amounts depends on aim of phosphorylation). Water was added to adjust the volume to 50 μl.

For preparative phosphorylation, 2-3 mg of peptide was dissolved in 1.5 ml of water and added to 450 μl 5x kinase buffer. The pH was adjusted to 7.0. 250 μl of

0.1 M ATP and 500 μl of A431 plasma membranes (~2 mg/ml) was added and then the reaction was allowed to proceed for 18 h at room temperature with continual mixing.

Isolation of Phosphorylated Peptides by Reverse Phase HPLC

One millilitre of buffer A (Buffer A: 0.08% trifluoracetic acid, 1% acetonitrile in water; Buffer B: 0.08% trifluoracetic acid, 90% acetonitrile) was added to the kinase reaction and mixed. This solution was then spun for 20 min at 10,000g to pellet the membranes. The supernatant containing the phosphopeptide was then loaded onto a Sep-Pak column (C_{18}) equilibrated with buffer A. The column was washed with 20 ml buffer A to elute ATP and then the peptide was eluted with 3 x 1 ml of 40% buffer B. The OD of the fractions was monitored at 268 nm and fractions containing peptide were pooled and then lyophilised to dryness (note that the phosphorylated Y751 peptide has essentially no absorption at 280 nm). The phosphopeptide was then separated from non-phosphorylated peptide using a 1090 HPLC system. For preparative separation a C_{18} column (Aquapore OD-300, 250 x 7 mm) equilibrated with 100% buffer A (214 nm (sen. 50 mV)/280 nm (sen. 200 mV) was used with a 2 ml/min flow rate. The peptide was dissolved in 200 μl HPLC grate water and then loaded via a 500 μl loop. The column was then washed for 10 min with 100% buffer A before eluting the peptide and phosphopeptide with a 30 min linear gradient 0 to 45% buffer B followed by 5 min linear gradient to 100% buffer B. Peak fractions were collected manually. The pool fractions were diluted with water, lyophilised and then stored at -20^{o}C.

Phosphoamino Acid Analysis of Phosphorylated Peptides

Peptides phosphorylated in the presence of [γ-^{32}P]ATP using either purified EGF receptor or A431 cell membranes were purified by C_{18} Sep-Pak column and HPLC as described above. This material was then hydrolysed at 110^{o}C for 1

h in 1 ml of 6 M HCI. One millilitre of HPLC grade water was added and the sample was centrifuged at 10,000g for 10 min to removed debris. The remaining supernatant was frozen and lyophilised to dryness. The pellet was resuspended in 2 ml of water, frozen and then lyophilised once more. This material was analysed by two dimensional thin-layer electrophoresis (essentially as described by Cooper et al, 1983).

Coupling of Peptides to Actigel Resin

Peptides were coupled to the matrix essentially as described by the manufacturers. Briefly, 500 μl (packed volume) of Actigel-ALD Superflow resin (Sterogene, CA, USA) was washed five times with 100 mM phosphate buffer (pH 7.8) (coupling buffer). Phosphorylated or non-phosphorylated peptide (1 mg) was dissolved in 200 μl of coupling buffer and added to the resin. $NaCNBH_3$ (coupling solution) was added to a final concentration of 100 mM and this was then mixed at 4°C for 6 h. The resin was washed with 10 column volumes of 500 mM NaCl and then incubated with 100 mM Tris-HCI (pH 8.0) for 1 h in the presence of coupling solution to block any unreacted sites on the resin. The resin was washed with 500 mM NaCl and finally with coupling buffer plus 500 μM vanadate and 0.02% NaN_3 and then stored at 4°C. Phosphopeptides bound to the Actigel matrix were stable for several months under these conditions.

Binding of Proteins to the Phosphopeptide Columns

Proteins were diluted in binding buffer (50 mM phosphate buffer (pH 7.2), 150 mM NaCl, 0.02% Triton X-100, 2mM EDTA and 200 μM sodium orthovanadate), mixed with the appropriate peptide affinity resin and then allowed to bind for 2h at 4°C with rotation. The column material was washed repeatedly (>6x) with 50 column volumes of the same buffer and then with various elution buffers containing NaCl, urea or detergents. Bound proteins were

either assayed for PI3-kinase activity or were removed from the column by boiling in SDS-PAGE sample buffer and then analysed by SDS-PAGE.

PI3-kinase Assay

PI3-kinase assays were carried out essentially as described in Whitman et al, (1987) in 50 μl containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM DTT, 0.5 mM EDTA, 5 mM $MgCl_2$, 100 μM ATP (plus 0.5 μCi [γ-^{32}P]ATP/assay), 1 mM PI plus soluble or column immobilised bovine brain PI3-kinase. Incubation was for 5 min at room temperature. The reaction was terminated by the addition of 100 μl of 0.1N HCl and 200 μl chloroform:methanol (1:1). The mixture was vortexed and then centrifuged to separate the phases. The upper phase was discarded and the lower organic phase washed with 80 μl of methanol: 1N HCl (1:1). After centrifugation the upper phase was again discarded and the lower phase evaporated to dryness. Reaction products were spotted on thin layer Silica gel 60 plates (pretreated with 1% oxalic acid, 1 mM EDTA in water:methanol (6:4)) and developed in chloroform:methanol:4 N ammonia (9:7:4).

Preparation of C-terminal Specific Antisera for p85α and p85β

C-terminal peptide antisera were prepared against the bovine C-terminal sequences determined by cDNA cloning (**Otsu et al, 1991**). The peptides TLAYPVYAQQRR for p85α and TLAHPVRAPGPGPPAAR for p85β were synthesized by FMOC chemistry and purified by HPLC. The peptides were coupled using gluteraldehyde to KLH and then injected into the lymph nodes of rabbits using methods described in Kypta et al, (1988). Positive antisera as determined by enzyme-linked immunoassay were affinity purified on specific peptide-Actigel affinity columns.

B. <u>Procedure and Results of Purification</u>

Preparation of Y751 Phosphopeptide Column

A 17 amino acid peptide which contains Y751 of the human PDGF-β receptor was chosen for synthesis in an attempt to inlcude all necessary sequence determinants following a survey of the known binding sites for the PI3-kinase (see Table 2 above; reviewed in Cantley et al, 1991). In addition to the peptide context of Y751 of the PDGF β-receptor, the sequences around Y315 of polyoma middle T (Talmage et al, 1989) and Y721 of the human CSF-1 receptor (Shurtleff et al, 1990) were also considered. Using the phosphorylation protocol described above, greater than 50% phosphorylation of the Y751 peptide was achieved using either purified human EGF receptor or A431 membranes as a source of protein-tyrosine kinase. The phosphorylated Y751 peptide could be clearly identified during reverse phase HPLC analysis, where it eluted approximately one minute earlier than the nonphosphorylated peptide, since it produced a strong 214 nm absorbance, but little or no 280 nm signal (Figure 1, panel A). Analysis of the absorption properties showed that phosphorylation of the Y751 peptide let to a shift in the absorption maximum from 280 to 267 nm (Figure 1, panel B). For large scale phosphorylations A431 membranes were the preferred source of protein-tyrosine kinase activity since they could be more easily generated. However, as the Y751 peptide contains two serines, as well as a single tyrosine residue, it was thought important to demonstrate that peptide was phosphorylated exclusively at the tyrosine residue. This was established by two separate methodologies; analysis of HPLC purified phosphopeptide by phosphoamino acid analysis or by protein microsequencing.

Phosphoamino acid analysis of the Y751 peptide, phosphorylated by either purified EGF receptor or A431 membranes, demonstrated that phosphorylation of the Y751 peptide was occuring exclusively at the tyrosine residue (Figure 1, panel C). Sequence analysis of the phosphorylated and non-phosphorylated peptides also

confirmed that both these peptides were 17 amino acids in length and that their sequences were identical except at cycle 10 where as expected no phenylthiohydantoin-Tyr derivative was observed for the phosphorylated peptide due to its modification.

Extended Purification of Bovine Brain PI3-kinase Using a Y751 Phosphopeptide Affinity Column

A 650-fold purification of PI3-kinase from bovine brain has recently been described (Morgan et al, 1990), and this same method was used except that the gradient for the second Mono Q column was extended to give two distinct peaks containing PI3-kinase activity (Figure 2, panel A). Both of these peaks (referred to hereafter as peak 1 (P1) and peak 2 (P2)) contained no PI kinase activity other than PI3-kinase activity as determined by HPLC analysis of deacylated product lipids (data not shown). However, both of these fractions still contained greater than 20 peptides detectable after SDS-PAGE gel analysis by silver staining (see Figure 2, panel A). The precise subunit composition of the active PI3-kinase complex was still a point of some contention, so an attempt was made to address this question by affinity purifying the PI3-kinase activity from these two Mono Q pools. The bovine brain PI3-kinase preparation was diluted 10-fold in binding buffer and allowed to bind batchwise to the Y751 phosphopeptide affinity resin for 4 h at 4°C. After washing the column extensively with binding buffer, those proteins which remained bound were eluted with SDS-containing buffers and examined by SDS-PAGE. Two major polypeptide species of approximate molecular weights 85 and 110 kD, which bound specifically to the phosphopeptide column, but not to an identical column prepared with unphosphorylated Y751 peptide, were identified in both Mono Q peaks and were observed to be quantitatively depleted from the bovine brain PI3-kinase preparation (Figure 2, panel B). Assaying the bound material, the presence of these two proteins appeared to

be sufficient to generate full PI3-kinase activity (Figure 3, lane 2). With fresh preparations of bovine brain PI3-kinase this column routinely removed >90% of the PI3-kinase activity present in Mono Q peaks 1 or peak 2 (c.f., Figure 3, lanes 2 and 3) following a single incubation. Neither the 85 and 110 kD proteins, nor PI3-kinase activity bound to a column with an equivalent concentration of non-phosphorylated Y751 peptide (Figure 3, lane 1) or to a column prepared with phosphotyramine, a phosphotyrosine analogue (data not shown). It should also be noted that binding of the PI3-kinase complex to the phosphopeptide column did not result in any apparent increase in the total enzyme activity present (Figure 3, c.f., lanes 2 and 6). In fact a slight decrease in activity was often observed, but this was judged to be due to the unstable nature of the highly purified enzyme which was found to be inhibited by traces of metal ions and reversibly inhibited by oxidation. It is estimated that this affinity purification step results in a 7-8,000-fold purification of PI3-kinase from bovine brain relative to the DEAE load (the overall purification achieved from tissue is in fact much greater).

Elution of p85, p110 and PI3-kinase Activity from the Phosphopeptide Column

Elution of the above PI3-kinase complex from the phosphopeptide column proved to be difficult to achieve due to the high affinity of the interaction. Kazlauskas and Cooper (1990) have previously noted that the binding of cellular p85 proteins to phosphorylated PDGF-receptor was stable to treatment with solutions containing ionic detergents, 2 M NaCl, 1 M urea or 0.2% SDS. The p85 subunits and PI3-kinase complex were also found to bind tightly to the Y751 phosphopeptide matrix, and were likewise not eluted under any of the above conditions. At 20°C the 85 and 110 kD proteins remained bound in the presence of either 2 M NaCl plus 0.5% Triton X-100, 5 M

NaCl, 6 M Urea, 50 mM phosphotyrosine or up to 1 mg/ml free Y751 phosphopeptide. Several alternative elution protocols were investigated without success. An elution medium supplied with the Actigel resin was able to remove both proteins but led to a complete loss of activity. Interestingly no suitable conditions could be established whereby the 110 kD, but not the 85 kD, subunit was released from the column suggesting that the interaction between the 110 and 85 kD subunits is of high affinity. Elution of bound proteins was routinely carried out by heating the resin to 80°C for 3 min in the present of 5 mM phosphate buffer (pH 7.0), 0.1% SDS, 0.1 mM DTT, 10% glycerol. The phosphopeptide column could be simply regenerated following elution by extensive washing in binding buffer (Figure 3, lanes 4 and 5) and could be successfully used at least ten times before any deterioration in binding was observed.

Analysis of the p85 and 110 kD Proteins Bound to the Phosphopeptide Column

The relationship of the 85 kD proteins observed to bind to the Y751 phosphopeptide column to the recently cloned p85α and p85β proteins was investigated using the polyclonal antisera generated against the predicted C-terminal 12 and 18 amino acids of p85α and p85β, respectively. Despite the high degree of overall sequence similarity between p85α and p85β, the amino acid sequence over this segment is significantly different and thus p85α or p85β specific antisera were expected to be produced. Furthermore the amino acid sequence corresponding to this peptide in p85α is completely conserved between human, bovine and murine cDNAs suggesting that antibodies generated against this sequence might be useful for studying the expression of different p85 proteins in species other than bovine (Escobedo et al, 1991b; Otsu et al, 1991; Skolnik et al, 1991). The corresponding region of p85β in species other than bovine is currently unknown.

The p85 antisera generated against these peptides could specifically immunoprecipitate the appropriate species of expressed recombinant p85 from either COS-1 or Sf9 cells but were not very efficient at immunoprecipitating PI3-kinase activity from either cell lines or from the partially purified bovine brain PI3-kinase preparation. However, these antisera were found to work well in Western blots. The data presented in Figure 4 shows that these two antisera specifically recognized expressed p85 proteins present in either COS cells or in Sf9 cells. Longer exposures also revealed the endogenous COS p85 protein(s), but no such proteins were detected in Sf9 cells with these antisera. No cross reactivity was observed even at high concentrations of the recombinant proteins suggesting that they are specific for p85α (Figure 4, panel A) and p85β (Figure 4, panel B) respectively. The ability of these antisera to interact with the appropriate p85 species was demonstrated to be completely blocked in the presence of the appropriate peptide used ot raise the antisera (Figure 4, panel C). The p85 species in the two peaks of bovine brain PI3-kinase activity which bound to the Y751 phosphopeptide column was found to react exclusively with the anti C-terminal antisera raised against the p85α specific sequence (Figure 4, panel A). Following immobilisation of the bovine brain PI3-kinase material on the Y751 phosphopeptide column, all the p85α immunoreactive material was bound to the column with none detectable by either silver staining or Western blot analysis in the supernatant (Figure 4, panel D).

For sequence analysis of the PI3-kinase complex, the 110 and 85 kD subunits were eluted from the column, following extensive stringent washing, by briefly boiling the resin in 5 mM phosphate buffer (pH 7.0), 0.1% SDS, 0.1 mM DTT, 10% glycerol. Preparation of both 85 and 110 kD proteins for digestion with lysylendopeptidase and subsequent sequence analysis were performed in accordance with the

protocol given hereinbefore. Amino acid sequence analysis of a lysylendopeptidase C digest of the p85 protein bound to be Y751 phosphopeptide column confirmed that the p85 protein present in both peak 1 and peak 2 from the mono Q column were identical to the previously cloned p85α (Otsu et al, 1991). No peptides corresponding to p85β were found in either peak. Extensive sequencing of the 110 kD protein affinity purified from both mono Q peak 1 and peak 2 material enabled the isolation of a novel cDNA (see below).

Specificity of Binding of the Purified Bovine Brain PI3-kinase

In order to evaluate the specificity of the Y751 phospopeptide column for purifying the PI3-kinase, other phosphopeptide columns were prepared using peptides based on the amino acid sequences which surround known protein-tyrosine kinase phosphorylation sites. Tyrosine 857 is the other major autophosphorylation site in the human PDGF β-receptor and has been shown to be required for the binding of GAP, but not for association with the PI3-kinase (Kazlauskas & Cooper, 1989, 1990; Kazlauskas et al, 1991). For a direct comparison with the Y751 peptide a 17 amino acid peptide centred around tyrosine residue 857 was synthesized (see Table 2 above). A comparison the proteins from baculovirus expressing p85α Sf9 cell lysate or from bovine brain PI3-kinase fractions from mono Q peak 1 (P1) and peak 2 (P2) binding to either the Y751 (panel A) or Y587 (panel B) phosphopeptide columns is shown in Figure 5. Whereas the baculovirus expressed p85α is observed to bind both columns to a similar extent, the 85 and 110 kD proteins from both peaks of activity are seen only to bind to the Y751 phosphopeptide column. Similarly, PI3-kinase activity is only found associated with the Y751 phosphopeptide column (Figure 7, panel B).

To determine whether this binding specificity could be

extended several other peptides were synthesized based on known tyrosine autophosphorylation sites (see Table 2 above). A shorter, 11 amino acid version of the Y751 peptide, termed Y751S, was also synthesized in an attempt to further refine the minimal SH2 recognition domain required. Two other peptides containing the YXXM motif were prepared, one based on the seqeunce around tyrosine 740 of the PDGF-β receptor, a second residue within the PDGF receptor kinase insert which may play a role in PI3-kinase binding (Escobedo et al, 1991a), and the second based around tyrosine Y1313 of Met, the hepatocyte growth factor receptor. To introduce a totally random sequence the synthetic peptide poly Glu:Ala:Tyr (6:3:1) was also phosphorylated and coupled to the Actigel matrix. Finally the peptides surrounding the two major phosphorylation sites from pp60^{c-src}, Y416 and Y527, were purchased and synthesized respectively. All peptides efficiently phosphorylated specifically on tyrosine residues using the EGF receptor and then were purified by HPLC as described above for the Y751 phosphopeptide.

Baculovirus expressed bovine p85α and p85β were chosen to test these columns (Otsu et al, 1991). Binding analysis was carried out under identical conditions to those previously established for the Y751 phosphopeptide column. Somewhat unexpectedly the baculovirus expressed p85 subunits bound to all phosphopeptide columns tested (see Figure 7, panels A and B). They did not however bind to identical columns containing non-phosphorylated peptides (Figure 6, panels A and B, lane 1 and data not shown). However when partially purified bovine brain PI3-kinase was applied to these columns it was found to bind exclusively to the phosphopeptide columns containing a YXXM motif (see Figure 7 and Figure 8, panel A).

That the Y751S phosphopeptide column appears to be as efficient at binding the active PI3-kinase complex as the longer Y751 phosphopeptide column suggests that the

consensus sequence recently proposed by Cantley et al, (1991) does indeed contain all the sequence data necessary for correct recognition by the PI3-kinase SH2 domain (Figure 8, panel B).

CLONING OF p110

C. Experimental Procedures

Materials

Restriction enzymes and DNA modification enzymes were obtained from standard commercial sources and used according to the manufacturer's recommendations. Oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer and used directly in subsequent procedures.

Cells

The SGBAF-1 cell line was established by transfection of bovine adrenal cortex zona faciculata cells with pSV3neo as previosly described for other cell types (Whitley et al, 1987). SGBAF-1 cells and COS-1 cells were maintained in Dulbecco's modified eagle medium (DMEM) containing a 10% foetal calf serum (FCS). Maintenance of Spodoptera frugiperda (Sf9) cells was carried out as described by Summers and Smith, 1987.

Protein Purification and Amino Acid Sequence Determination

The purification of the p85α and p110 proteins by chromatography on a peptide affinity column corresponding to amino acids 742-758 of the kinase insert region of the human PDGF-β receptor has been described above. The method used for the final purification of p110 for amino acid sequence analysis was in accordance with the Protocol given hereinbefore. This procedure was carried out on three separate PI3-kinase preparations. A fourth preparation was eluted from the matrix as before and

boiled for 5 min. After cooling, the sample was diluted with 25 mM Tris-HCl, pH 8.8 and digested directly with lysylendopeptidase for 72 h at 30°C. Peptides were separated as above. Peptide sequences were determined using a modified Applied Biosystems 477A automated pulse-liquid sequencer.

mRNA Isolation and cDNA Cloning

Total RNA was isolated fromthe SGBAF-1 by the method of Chirgwin et al. (1979) and poly(A)$^+$ mRNA selected by chromatography on oligo-dT cellulose (Maniatis et al., 1982). An oligo-dT primed cDNA library of 5 x 10^6 primary recombinants was constructed in lambda Uni-Zap (Stratagene) from 5 μg of this mRNA using the Stratagene Uni-Zap cDNA cloning system. The construction of the total bovine brain cDNA library in lambda Uni-Zap has been described previously (Otsu et al, 1991).

Library Screening and Hybridizations

The unamplified SGBAF-1 cDNA library (10^6 recombinants) was plated on E. coli K12 PLK-F′ (Stratagene) at a density of 10^5 plaques per 15 cm dish and lifts taken in duplicate onto nitrocellulose membranes (Millipore). For screening, filters were prehybridized for at least 1 h at 42°C in 6 x SSPE, 0.5% SDS, 10 x Denhardt's solution, 100 μg ml^{-1} denatured sonicated herring sperm DNA (Sigma). Hybridization was carried out in the same solution containing 10 ng ml^{-1} radiolabelled oligonucleotide. Oligonucleotides used were: Peptide N (MDWIFHT) 5′-AA(G/A)ATGGA(T/C)TGGAT(C/T/A)TT(T/C)CA(T/C)AC-3′); Peptide J (D D G Q L F H I D F G H F) 5′-GATGATGGCCA(G/A)CTGTT(T/C)CA(T/C)AT(T/A)GA(T/C)TTTGGCCA(T/C)TT. Oligonucleotides were labelled with ^{32}P at the 5′ end in a 20 μl reaction containing 100 ng oligonucleotide, 1 x kinase buffer (Promega), 0.1 mM spermidine, 5 mM dithiothreitol, 100 μCi [γ-^{32}P]ATP (5000 Ci mmol^{-1}, Amersham) and 2 μl (20 U) T4 polynucleotide kinase (Amersham). Filters

were washed in 6 x SSC, 0.1% SDS at room temperature and then subjected to autoradiography using Kodak XAR film. Hybridizing clones were plaque-purified and rescued as plasmids according to the manufacturers instructions.

Characterization of cDNA Clones

Sequencing was carried out by the chain termination method using the Sequenase system (United States Biochemicals). Clones for sequencing were obtained by directed cloning of restriction fragments into M13 mp18 and mp19 vectors (Yanisch-Perron et al., 1985) and by making a series of exonuclease III mediated deletions (Henikoff, 1984; Pharmacia Exonuclease III deletion kit). DNA sequences were analysed on a MicroVAX computer using the Wisconsin (UWGCG: Devereux et al., 1984) sequence analysis package.

RACE PCR

RACE PCR was carried out essentially as published previously (Frohman et al., 1988; Harvey and Garlison, 1991). Briefly, first strand cDNA primed with random hexamers (Amersham) was synthesized from 1 μg of SGBAF-1 cell mRNA using the Stratagene first strand cDNA synthesis kit. First strand cDNA was isolated by isopropanol precipitation and tailed with oligo-dA using terminal deoxynucleotidyl transferase (BRL). PCR was performed using oligo 2224 (5′-AATTCACACACTGGCATGCCGAT) and adaptor-dT (5′-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTTT) as primers using a Perkin Elmer/Cetus Tap polymerase PCR kit (conditions: 94°C 1 min, 35°C 1 min, 72°C 2 min, 30 cycles). Products were fractionated on a 1.5% low melting point agarose gel and visualized by staining with ethidium bromide. The gel was sliced into 6 bands (size range 150-2000 bp) and DNA isolated from each gel slice. A further round of PCR was performed on this DNA using oligonucleotide 2280 (5′-TTTAAGCTTAGGCATTCTAAAGTCACTATCATCCC) and adaptor (5′-GACTCGAGTCGACATCGA) as primers (conditions: 94°C 1 min, 56°C 1 min, 72°C 2 min, 35 cycles). Products were fractionated on an agarose gel and visualised by staining with ethidium

bromide. A band 250 bp shorter than the size of the DNA in the gel slice used for the PCR was expected. An intensely staining band of 350 bp obtained from the ~600 bp gel slice was excised, digested with Hindlll and Sall and ligated into Bluescript KS- digested with Hindlll and Xhol to give plasmid pBS/race. Two independent inserts were completely sequenced.

Southern Transfer Hybridizations

High molecular weight DNAs were isolated from cell lines by standard techniques (Maniatis et al, 1982). DNAs were digested with restriction endonucleases, fractionated through 0.5% agarose gels and transferred to nitrocellulose (BA85, Schleicher and Schuell) as described in Maniatis et al (1982). Prehybridization was carried out in 1 M NaCl, 10 x Denhardt's solution, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.1% SDS and 100 μg ml^{-1} denatured sonicated herring sperm DNA at 65°C. Hybridization was carried out overnight in the same solution containing 20 ng ml^{-1} radiolabelled probe fragment (0.88 kb Xbal-Psti fragment: Probe a, Figure 9, lower panel) of specific activity >10^{8} dpm μg^{-1}). Probe fragments were isolated from agarose gels be electroelution (Maniatis et al, 1982) and labelled by nick translation (Rigby et al, 1977) using [α-^{32}P] dATP(>3000 Ci mmol-1, Amersham). Membranes were washed extensively in 0.1 x SSC, 0.1% SDS at 68oC or at 50°C in 0.5 x SSC, 0.1% SDS to detect related sequences, and subjected to autoradiography with Kodak XAR film.

Northern Transfer Hybridizations

Poly(A)$^{+}$ RNA from total bovine brain or the SGBAF-1 cell line was modified with DMSO and glyoxal and fractionated on a 0.9% agarose gel run in 10 mM phosphate buffer (pH 7.5) (Maniatis et al, 1982). Nucleic acid was transferred to nylon membranes (Hybond-N, Amersham) and filters baked dry. Prehybridization was carried out at 60°C in 50% formamide, 5 x SSPE, 5 x Denhardt's solution, 0.2% SDS, 200 μg ml^{-1} denatured sonicated herring sperm DNA and 200 ug ml^{-1} yeast RNA. Hybridization was carried out in the same solution containing 1 x 10^{7} cpm ml^{-1} antisense RNA probe. Probe was prepared by in vitro

transcription of a 2 kb fragment (nucleotides 598-2608; Probe b, Figure 9, lower panel) subcloned in pSPT19 (Boehringer), using SP6 RNA polymerase (Amersham) and [α^{32}-P] UTP (Amersham) according to the manufacturers conditions. Membranes were washed in 0.1 x SSC, 0.1% SDS at 60°C. Filters were treated with 1 μg ml^{-1} RNAase A (Sigma) in 2 x SSC for 15 min at room temperature and the filter rinsed at 50°C in 0.1 x SSC. Filters were then subjected to autoradiography against Kodak XAR film at -70°C.

PCR Determination of p85α and p110 mRNA

For p85α 125 ng of poly (A)$^+$ RNA was reverse transcribed with 2.5 units rTth DNA polymerase (Perkin-Elmer-Cetus) at 70°C for 10 min in a 10 μl reaction containing 10 mM Tris-HCl (pH 8.3), 90 mM KCl, 1 mM MnCl, 0.5 mM dNPT mixture and 1.2 μM antisense primer (5'-CAGGCCTGGCTTCCTGT). For DNA polymerization the reaction volume was adjusted to 50 μl by adding a single mix giving the following final concentrations: 5% (v/v) glycerol, 10 mM Tris-HCl (pH 8.3), 100 mM KCl, 0.75 mM EGTA, 0.05% (v/v) Tween 20, 2 mM $MgCl_2$, 0.24 μM sense primer (5'-AACCAGGCTCAACTGTT). PCR was then performed under the following reaction conditions: 92°C 1 min, 58°C 1 min, 72°C 1 min for 25 cycles on a Perkin Elmer-Cetus DNA thermal cycler.

Conditions for p110 were similar except concentration of the antisense primer (5'-TGCTGTAAATTCTAATGCTG) was increased to 4.8 μM during the reverse transcription step. DNA polymerisation conditions were the same except the final $MgCl_2$ concentration was increased to 2.5 mM and both primers (sense primer = 5'-GTATTTCATGAAACAAATGA) were present at a final concentration of 0.96 μM. Taq DNA polymerase (Promega) was also added at 0.03 U μl^{-1}. PCR was performed as follows: 92°C 30 sec, 54°C 5 sec, 72°C 30 sec for 35 cycles. 20 μl of each reaction was run on a 3% agarose gel (Maniatis et al, 1982) and visualised by staining with ethidium bromide.

Antibodies and Immunoprecipitations

For the preparation of the anti C-terminal p110 antiserum,

peptide CKMDWIFHTIKQHALN was synthesized by FMOC chemistry and purified by HPLC. It was then coupled to KLH using glutaraldehyde, and injected into the lymph nodes of rabbits using methods described in Kypta, R M et al., (1990), Cell 62, 481-492. Positive antisera as determined by enzyme-linked immunoassay were affinity purified on specific peptide-Actigel affinity columns. Anti-p85α (Otsu et al, 1991) and anti CSF-1 receptor (Ashmun et al., 1989) antisera are previously documented. Immunoprecipitations were carried out as described in Otsu et al., 1991.

PI3-kinase Assay

The assay for PI3-kinase activity was carried out as described by Whitman et al. (1985).

Expression of p110 in Sf9 Cells

To clone the p110 coding region into the baculovirus transfer vector p36C (Page, 1989) a Sau 3A1 site (GGATCA) present 10 nucleotides upstream from the initiation codon (see Figure 9) was changed to a BamH1 (GGATCC site by PCR mediated mutagenesis. Briefly, a sense oligonucleotide substituting C for A at position 6 of the Sau3A1 site was used in a PCR reaction with an antisense primer comprising nucleotides (102-124) of the p110 sequence (see Figure 9) using Vent polymerase (New England Biolabs). Template DNA was random-primed first strand cDNA prepared from SGBAF-1 cell mRNA as described above; PCR conditions: 94°C 1 min, 50°C 1 min, 72°C 2 min, 35 cycles. The PCR product was digested with BamH1-EcoN1 and a 118 bp fragment isolated from a low melting point agarose gel. This BamH1-EcoN1 fragment was cloned into p110/2.2 digested with BamH1 (present in vector sequences) and EcoN1 (nucleotide = 108) giving plasmid p110/(BamH1). The BamH1-EcoN1 fragment of p110/(BamH1) was sequenced and found to agree with that previously determined. A 3.4 kb BamH1-Kpn1 (Kpn1 site present in the vector) fragment was isolated from p110/(BamH1) and ligated into p36C baculovirus transfer vector (Page, 1989) previously digested with the same enzymes. Recombinant viruses were obtained as described in Summers and Smith

(1987). Sf9 cells were infected at a multiplicity of infection of 10 with recombinant viruses in IPL-41 media supplemented with 10% FCS. Cells were harvested and lysed 2 days post-infection in EB lysis buffer (20 mM Tris-HCl (pH 7.4), 50 mM NaCl, 50 mM NaF, 1% NP40, 1 mM EDTA, 500 μM sodium orthovanadate, 2 mM PMSF, 100 Kallikrein inhibitor units of Aprotinin ml^{-1}) (Kazlauskas and Cooper, 1989) and lysates were analysed by immunoappreciation.

Association of p110 and p85α with CSF-1 Receptor

This assay was performed essentially as described by Kazlauskas and Cooper (1990). Sf9 cells were infected as already described and lysed 48 h post-infection in EB lysis buffer. CSF-1 receptor was immunoprecipitated from Sf9 cells

and collected on Protein A-Sepharose beads. The immunocomplex was then subjected to extensive washing (3 times with EB lysis buffer, twice with kinase buffer; 50 mM HEPES (pH 7.4), 150 mM NaCl, 0.02% Triton X-100, 12 mM $MgCl_2$, 2 mM $MnCl_2$, 10% glycerol, 500 μM sodium orthovanadate) and the receptor phosphorylated for 15 min at 20°C with ATP. The precipitates were then washed again to remove free ATP and incubated for 2 h at 4°C with cell lysates prepared from Sf9 cells infected with viruses expressing (i) p85α, (ii) p110 or (iii) co-infected with viruses expressing p85α and p110. The immune complexes were washed and assayed for associated PI3-kinase activity.

Expression of p85α and p110 in COS-1 Cells

For transient expression of p85α in COS-1 cells the coding region for p85α was cloned into the adenovirus late promoter based expression vector pMT2 (Kaufman et al, 1989) as previously described (Otsu et al, 1991). For expression of the p110 cDNA plasmid pSG5-p110 was constructed as follows. The 3.4 kb BamH1-Hindlll fragment from cDNA p2.1 was ligated into pSG5 (Stratagene) cut with BamH1 and Bglll, the Hindlll and Bglll overhangs of p2.1 and pSG5 respectively, being filled in with Klenow polymerase. This gave construct pSG5.2.

Plasmid pBS/race (above) was digested with EcoRl and Hindlll, the 350 bp band gel purified by electroelution (Maniatis et al, 1982) and further digested with Sau3Al and Bsml. This mixture was then added to the gel purified Bsml-BstMl fragment from p2.1 and ligated in a three fragment ligation to pSG5.2 digested with BamHl and BstXl. 5 μg of each DNA was transfected into 10 cm dishes of 80% confluent COS-1 cells using Lipofectin (BRL) under conditions suggested by the manufacturers. Lysates were analysed by immunoprecipitation with anti-p85α polyclonal antiserum or with anti-p110 C-terminal peptide antiserum. Immunocomplexes collected on Protein A-Sepharose beads were analysed either on 10% SDS-PAGE gels followed by autoradiography or subjected to in vitro PI3-kinase assays as described.

D. Results of Cloning

cDNA Cloning and Deduced Amino Acid Sequence of p110

Initially, an oligo(dT) primed bovine brain cDNA library (Otsu et al, 1991) was screened with oligonucleotide probes made against peptides J and N (see Figure 9). No hybridizing clones were detected. Therefore, a new cDNA library of 5 x 10^6 primary recombinants was constructed from mRNA isolated from a pSV3neo transfected bovine adrenal cortex zona fasciculate cell line (SGBAF-1), which was known to contain PI3-kinase activity (Otsu et al, 1991). Screening of 1 x 10^6 primary recombinants from this library with the same oligonucleotides led to the detection of 66 clones positive with both probes. Twenty overlapping clones were characterized and found to possess inserts from 1-4 kb. The clone with the longest insert representing coding sequence (clone p110/2.1) was completely sequenced. This revealed a potential open reading frame (ORF) of 1053 amino acids with a predicted molecular weight of 123 kD. The ORF contained all the sequenced peptides, but was not preceded by in-frame stop codons. Since the predicted size of the p110 protein from SDS gels is 110 kD, it was possible that the protein could initiate from an internal methionine within this ORF.

Expression studies carried out in COS-1 cells using methionines 16, 30, 123 and 130 as potential start codons (initiation at Met 123 would give rise to a protein of 110 kD) did not lead to the syntheses of a protein corresponding to p110 or any augmentation of PI3-kinase activity in these cells. This suggested p110/2.1 is missing 5′ coding sequence and that either p110 protein runs anomalously on SDS-PAGE gels or that it is synthesized as part of a larger precursor molecule. Characterization of the remaining 46 positive clones initially isolated, showed that all had inserts shorter than that in clone p110/2.1. To further extend the p110/2.1 cDNA in the 5′ direction a RACE (rapid amplification of cDNA ends) polymerase chain reaction (PCR) (Frohman et al, 1988; Harvey and Garlison, 1991) was used. Two independent products which extended the known nucleotide sequence were characterized (see Figure 9, lower panel). The nucleotide and deduced amino acid sequences for the coding region of the composite cDNA are presented in Figure 9. The putative initiation codon is preceded by an in-frame stop codon and occurs in a Kozak consensus sequence (Kozak, 1987) for the initiation of translation (data not shown). The deduced amino acid sequence encodes a protein of 1068 amino acids with a calculated relative molecular mass of 124,247.

Analysis of the p110 Nucleotide and Deduced Amino Acid Sequences

The coding region of the cDNA for p110 is extremely A+T rich (G+C content = 39.3%) which is reflected in the failure to use codons TCG (Serine) and GTC (Valine). When the p110 amino acid sequence was compared with sequences in the Swissprot and NBRF protein databases, significant homology was found to only one protein, Vps34p (Figure 10). This is a rare 100 kD protein from Saccharomyces cerevisiae involved in the sorting of proteins into the yeast vacuole and in the vacuole morphogenesis during budding (Herman and Emr, 1990). A search of the p110 sequence for amino acids conserved in the active sites of kinases, reveals G_{842}, K_{863}, D_{916}, N_{921}, and the DFG triplet at residues 933-935 (these residues are marked in

Figure 2B) which might be homologous to G_{52}, K_{72}, D_{166}, N_{171} and the DFG triplet at residues 184-186 in cAMP-dependent protein kinase (Knighton et al, 1991a,b). Equivalent residues are present in Vps34p and are also marked in Figure **X**. The glycine rich P-loop (Saraste et al., 1990), found in many kinases (Hanks et al., 1988), does not appear to be present in either p110 or Vps34p.

Genomic Southern Blot Analysis of p110 Genes

Given the occurrence of at least two forms of p85 (Otsu et al, 1991), Southern blot analysis was used to analyse the number of p110 related genes which occur in genomic DNA isolated from bovine, human and rat sources. The analysis clearly provides evidence for a second, closely related, gene in rat and human genomic DNA (e.g. compare Figure 11A lanes 4 and 9 with Figure 11B lanes 4 and 9). For bovine DNA there appear to be no hybridization signals removed by washing at higher stringency (compare Figure 11A lanes 1, 2 and 3 with Figure 11B lanes 1, 2 and 3). However, it is possible that a related gene exists in bovine DNA, but, either it does not cross-hybridize under the conditions used, or it is too similar in sequence to be detected by differential washing.

Expression of p110 Cells and Tissues

A northern blot analysis carried out on mRNA isolated from the SGBAF-1 cell line and total bovine brain is shown in Figure 12A. Both mRNA samples contain major p110 specific transcripts of 4.8 kb and 9 kb, although there is substantially more p110 message present in mRNA isolated from SGBAF-1 cells (Figure 12A, lane 2) than that isolated from total bovine brain (Figure 12A, lane 1). A PCR based study was performed to examine the distribution and conservation of p110 mRNA in cell lines and tissue from several species. Amplification of a p110 specific fragment is seen for three human mRNAs (218 bp; Figure 12B lanes 1, 2 and 3) and two bovine mRNAs (212 bp; Figure 12B, lanes 5 and 6). Similar sized fragments are amplified from cell lines of simian and porcine origin (Figure 12B, lanes 4 and 7, respectively), indicating the existence of

a p110 homologue in these species. An additional band of 300 bp is amplified from bovine brain mRNA (Figure 12B, lane 5) and its identity is currently being investigated. Since PI3-kinase activity may reside in a p85α/p110 complex (Carpenter et al, 1990; Otsu et al., 1991; Shibasaki et al., 1991), some of these cell lines were examined to see whether messages for p85α and p110 are co-expressed. Amplification of a p85α specific 190 bp fragment is seen for the three human omission (Figure 12C, lanes 1, 2 and 3) cell lines and one simian (Figure 12C, lane 4) cell line analysed. Thus, at least in these four cell lines, messages for p85α and p110 are co-expressed.

p110 cDNA Encodes a Protein of Apparent Molecular Weight 110 kD which Possesses PI3-kinase Activity

To demonstrate that the p110 cDNA encodes the 110 kD subunit of PI3-kinase, it was expressed in the baculovirus expression system (Summers and Smith, 1987). Immunoprecipitation with an anti-p110 antiserum from Spodoptera frugiperda (Sf9) cells infected with the p36C-p110 virus revealed a novel protein of apparent molecular weight 110 kD (Figure 13A, lane 4) which co-migrated with the p110 PI3-kinase subunit purified from bovine brain. No such protein was seen in anti-p110 immunoprecipitates prepared from cells infected with a control wild-type virus (Figure 13A, lane 2). This baculovirus expressed p110 was used to examine whether p110, alone, possesses catalytic activity or whether a p85α/p110 complex is required. When assayed, p110-containing immunoprecipitates were found to possess significant levels of PI3-kinase activity (Figure 13B, lane 4), the identity of the lipid product being confirmed as PI(3)P by HPLC analysis. No activity was detected in anti-p110 immunoprecipitates prepared from control infected cells (Figure 13B, lane 2). These results clearly demonstrate that the p110 subunit of PI3-kinase is sufficient for catalytic activity.

p110 Expressed in Insect Cells Forms a Stable Complex with p85α

Since PI3-kinase purified from bovine brain is a complex of p85α and p110, the ability of p85α and p110 expressed in insect cells to reconstitute an active p85α/p110 complex was examined. Baculoviruses expressing either p85α (pAcC4-p85α; Otsu et al, 1991) or p110 (p36C-p110) were infected separately, or together, into Sf9 cells and expressed proteins analysed as described in experimental procedures. Immunoprecipitates of p85α alone (Figure 13A, lane 3) were inactive in a PI3-kinase assay (Figure 13B, lane 3) as previously demonstrated (Otsu et al, 1991). In double infection experiments, both p85α and p110 were detected in either anti-p85α (Figure 13A, lane 5) or anti-p110 (Figure 13A, lane 6) immunoprecipitates. As neither subunit-specific antiserum recognises the other subunit (see Figure 15A, lane 3; Figure 15C, lane 2), the simplest interpretation of this data is that, when expressed in Sf9 cells, p110 and p85α (Figure 13B, lane 5) or the anti-p110 antisera (Figure 13B, lane 6) were both active. Neither antiserum immunoprecipitated endogenous PI3-kinase activity from Sf9 cells infected with wild-type virus (Figure 13B, lanes 1 and 2).

PI3-kinase Activity Expressed in Sf9 Cells Can Associate with the Activated CSF-1 Receptor

PI3-kinase activity has been shown to associate with many activated PTK receptors, but particularly well studied have been those receptor PTKs possessing a kinase insert region, e.g., PDGF-β receptor (Coughlin, S R et al., (1989), Science 243, 1191-1193 and the CSF-1 receptor (Varticovski et al, 1989; Shurtleff et al, 1990). An in vitro association assay (Kazlauskas and Cooper, 1990) was used to study the association of PI3-kinase activity expressed in insect cells with the activated CSF-1 receptor. Figure 14 shows that baculovirus expressed PI3-kinase activity can associate with the CSF-1 receptor, but only from an Sf9 cell lysate containing both p85α and p110 (Figure 14, lane 2), and only

when the receptor has been phosphorylated prior to incubation with Sf9 cell lysate (compare Figure 14, lanes 2 (+ ATP) and 3 (- ATP)). No PI3-kinase activity associates with CSF-1 receptors incubated with Sf9 cells lysates containing p85α alone (Figure 14, lane 4) or p110 alone (Figure 14, lane 5). No activity is found associated with the CSF-1 receptor immunoprecipitated from Sf9 cells (Figure 14, lane 1). Thus, PI3-kinase subunits expressed in insect cells can be used to reconstitute an active p85α/p110 complex that binds to a phosphorylated PTK receptor.

Expression of PI3-kinase in COS-1 Cells

The results shown above were all obtained from expression studies carried out in insect cells. In order to study p110 and its interaction with p85α in a mammalian cell system, transient co-expression studies in COS-1 cells were performed. The p110 cDNA was cloned into the SV40 based expression vector, pSG5 (giving plasmid pSF5-p110) and transfected into COS-1 cells, either alone or together with a p85α expression construct, pMT2-p85α (Otsu et al., 1991). To enable proteins to be more easily visualised transfected COS-1 cells were metabolically labelled with ^{35}S-methionine for 3-4 h prior to lysis. Radiolabelling at this time results in preferential labelling of proteins synthesized from transfected constructs. Cell lysates were immunoprecipitated with either anti-p85α (Figure 15, panels A and B) or anti-p110 antisera (Figure 15, panels C and D). Immunoprecipitated proteins were either visualised by autoradiography following fractionation on SDS-PAGE gels (Figure 15, panels A and C) or subjected to an in vitro PI3-kinase assay (Figure 15, panels B and D).

Transfection of pMT2-p85α resulted in a significant elevation of p85α over the background level due to endogenous simian p85α - compare Figure 15A, lanes 2 and 4 with Figure 15A, lane 1. In p85α/p110 co-transfectants, the anti-p85α antiserum co-immunoprecipitates p85α and p110 (Figure 15A, lane 4), demonstrating the existence of a p85α/p110 complex. When assays for PI3-kinase activity were performed on the anti-

p85α immunoprecipitates, enhanced activity (10 fold over the background due to endogenous simian PI3-kinase) was only detected with immunoprecipitates which contained both p85α and p110 (compare Figure 15B, lane 4 with Figure 15B lanes 1, 2 and 3). These results demonstrate that in COS-1 cells, as in Sf9 cells, the p110 cDNA directs the synthesis of a protein of molecular weight 110 kD, which associates with p85α to give a p85α/p110 complex that possesses PI3-kinase activity.

However, when proteins were immunoprecipitated from the same lysates with the anti-p110 antiserum and PI3-kinase assays performed, the results were surprising. As expected. the anti-p110 antiserum immunoprecipitated p110 from cells transfected with pSG5-p110 (Figure 15C, lane 3). However, in addition, it would only immunoprecipitate free p110 from lysates prepared from cells co-transfected with p85α and p110 (Figure 15C, lane 4) even though p85α/p110 complex was present in these lysates (Figure 15A, lane 4). When assayed for PI3-kinase activity, no activity above that present in control immunoprecipitates (Figure 15D, lanes 1 and 2), was present in p110 containing immunoprecipitates prepared from either p110-transfected (Figure 15D, lane 3) or, p85α and p110 co-transfected, cells (Figure 15D, lane 4). Thus, the anti-p110 antiserum is capable of immunoprecipitating p110 from cell lysates of both infected Sf9 cells (Figure 13A, lane 4) and transfected COS-1 cells (Figure 15C, lane 3), but only the immunoprecipitates prepared from Sf9 cell lysates possess elevated levels of PI3-kinase activity (compare Figure 13B, lane 4 and Figure 15D, lane 3). Also, the anti-p110 antiserum immunoprecipitates the p85α/p110 complex when expressed in Sf9 cells, but not when expressed in COS-1 cells.

As indicated above, analysis of the cloned p110 cDNA shows it to encode a protein of 1068 amino acids with a calculated molecular weight of 124 kD. The reason for the difference in size between the calculated (124 kD) and observed molecular weight value 110 kD is unclear, but it is known that many proteins migrate anomalously on SDS-PAGE gels. Expression of

the protein encoded by this ORF in Sf9 cells, COS-1 cells, reticulocyte lysate and E. coli all result in the production of a protein of apparent molecular weight 110 kD.

The deduced amino acid sequence of p110 contains all the peptide sequences determined by protein sequence analysis. Since the peptides were obtained from a lysylendopeptidase digestion, it is expected that they should all be preceded by an arginine residue. This is true in every case, except for peptide A which is preceded by an arginine residue (Arg 162). Nucleotide sequence data obtained from another cDNA clone covering this region confirms the presence of an arginine residue in this position. Thus, it seems likely that cleavage at this site by lysylendopeptidase results from a sequence polymorphism.

When a database search was performed on the p110 sequence no significant homology was detected with any proteins known to be involved in inositol lipid metabolism. However, as noted, p110 did show significant homology throughout its C-terminal half to the Saccharomyces cerevisiae protein Vps34p. The possibility that Vps34p is a yeast PI-kinase is currently being investigated. If p110 and Vps34p are homologous proteins then it is interesting to speculate that p110 might also be involved with protein targeting and/or vesicular transport. PI3-kinase activity has previously been implicated in vesicle mediated responses in higher eukaryotes. Hence, PI3-kinase activity is seen to increase following stimulation of platelets with thrombin (Kucera and Rittenhouse, 1990) and neutrophils with f-Met-Leu-Phe (Traynor-Kaplan et al, 1988). In both cases, ligand stimulation promotes the fusion of vesicular structures necessary for the biological response. A role for PI3-kinase in intracellular vesicles following the activation of PTKs has also been suggested (Cantley et al, 1991; Kelly et al, 1992).

Southern blotting data suggests there may be two genes for PI3-kinase in rats and humans. Evidence for the existence of

a second gene in rat DNA is also provided by the results of Carpenter et al., (1990), who identified two forms of p110 in their purified PI3-kinase preparation. In situ hybridization confirms the presence of two closely related sequences in human DNA, although one could be a pseudogene. Two forms of p85 (p85α and p85β) have been characterized (Otsu et al, 1991), although only p85α is found associated with p110 in PI3-kinase from bovine brain. It is possible to speculate that p85β associates with a second form of p110.

Although, at present, the function of the 3-phosphorylated phosphoinositides produced by PI3-kinase is unclear, the availability of expression systems which allow their generation will aid in the determination of their function.

Example 2

Using the bovine cDNA probe constituted by the XbaI-PstI fragment of the sequence of Figure 9 (probe a, bottom panel) and genomic DNAs from several species, Southern blot analyses prove positive against the bovine probe in the following species:- bovine (calf thymus), human (HeLa cells), rat (liver), simian (COS cells), porcine (ZNR cells), chicken (from Promega), and Xenopus (liver).

The human cDNA was isolated from a cDNA library, made from mRNA isolated from the human cell line KG1a using standard techniques. The probe was a partial cDNA from the second half of the bovine p110 cDNA. The probe was labelled with ^{32}P and hybridised overnight to the library filters at 65°C in 1M NaPi, 7% SDS buffer. The filters were washed in 2xSSC at 50°C, and exposed to X-ray film at -70°C. The nucleotide sequence is shown in Figure 16 together with the corresponding amino acid sequence. The human p110 sequence has 95% homology to the bovine p110 sequence at the DNA level and is 98% identical at the protein level (Figures 17 and 18). The protein sequence is shown in Figure 19. Primers (357) AAG GAT CAG AAC AAT GCC T and (416) AGG CTT TCT TTA GCC ATC A were

used to amplify, using RT-PCR (94°C 30 sec 50°C 30 sec, 72°C 60 secs; for 35 cycles) the partial sequence of a highly related p110 gene (p110-11). P110-11 has 96% nucleotide homology to p110 (sequence not provided).

Two novel cDNAs related to p110 have been cloned. Degenerate primers were designed to conserved sequences between human p110 and the related yeast gene VPS34 (Sense (GDDLRQD) 5′ GGN GAT/C GAT/C T/C TA/G CGN CAA/G GA-3′ antisense (FHIDFGHF) 5′A/GAA A/GTG ICC A/GAA A/GTC A/G/TAT A/GTG A/GAA-3). These were used in RT-PCR reactions using mRNA from the human cell lines MOLT4 and U937 (94°C 30 sec, 50°C 30 sec, 72°C 30 sec for 35 cycles). Two novel cDNA's, PITR-c and PITR-f, related to p110, were isolted. The PITR-c nucleotide sequence is shown in Figure 20. This gene is highly related to the yeast gene VPS34, the VPS34 protein is involved in the protein sorting from the golgi to the vacuole and has an intrinsic PI3-kinase activity. The PITR-f nucleotide sequence is shown in Figure 21 and is more similar to p110 than PITR-c and is likely also to possess PI3-kinase activity. The alignment of human p110, the human PI3-kinase related genes PITR-c and PITR-f and the yeast PI3-kinase VPS34 are shown in Figure 22. The amino acids conserved in 3 or more of the proteins are shown in the upper case.

The interation of the p85 and p110 subunits of PI3-kinase are thought to be required for the activity of the kinase in mammalian cells. Thus inhibiting the interaction between the subunits could provide a means of inhibiting the activity of this signal transduction pathway. In order to design reagents to p110 which will block the interaction, it is useful to define the region of p110 which binds to the p85 subunits. To do this a series of mutants were constructed which express various domains of the p110 protein (Figure 23B). These fragments were expressed as GST fusion proteins in bacteria. The proteins were then bound to a glutathione sepharose column (Pharmicia) according to the manufacturer's instructions (Panayotou G et al (1992) EmboJ 11:4261-4272). The ability of

these protein fragments to bind the p85 subunits was assessed by the ability of the column specifically to retain p85 subunits purified from baculovirus (Otsu et al(1991) Cell 65:91-104). As shown in Figure 23A, only p110-N ($\alpha\alpha$1-128) was capable of binding the p85α and β subunits. To further characterise the binding domain, deletion mutants and PCR fragments were made from the p110-N fragment as shown in Figure 24. The results in Figure 25 demonstrate that a domain containing amino acids 19-110 of human p110 is sufficient to associate with the p85 subunits. Removal of a further 20 amino acids from either the amino or carboxy termini led to loss of binding activity. Now that this domain has been identified it allows the design of specific peptides, antibodies or small molecules which can inhibit the interaction between the subunits.

The invention includes a human PI3-kinase p110 subunit sequence comprising amino acids 19 to 110 of human p110, or an amino terminal truncated or carboxy terminal truncated derivative thereof having less than 20 amino acids deleted from the amino terminal or carboxy terminal end, respectively, but which is capable of binding to a PI3-kinase p85 subunit; and also included is a method of inhibiting p85 and p110 mammalian PI3-kinase subunit interaction, which comprises utilizing a molecule which blocks the binding domain located between amino acids 19 and 110 of human p110.

The invention further provides the use of a sequence or derivative as defined above in screening for a therapeutic or prophylactic agent which operates by inhibiting interaction between p85 and p110 mammalian PI3-kinase subunits.

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Claims:

1. An isolated polypeptide which possesses PI3-kinase activity when produced by recombinant production in insect cells.

2. A polypeptide derivative of a polypeptide as defined in claim 1 and which has PI3-kinase activity and binds, when associated with a p85 mammalian PI3-kinase subunit, to a phosphopeptide which includes the YXXM motif, the tyrosine being phosphorylated.

3. A polypeptide as claimed in claim 1 or claim 2 and which is capable of association with p85 subunits of mammalian PI3-kinases to produce active complexes.

4. A polypeptide having the amino acid sequence of Figure 9, or exhibiting significant sequence homology with the amino acid sequence of Figure 9 and possessing PI3-kinase activity.

5. A polypeptide as claimed in any one of the preceding claims and which is of human origin.

6. An antibody to a polypeptide as defined in any one of the preceding claims, optionally monoclonal.

7. A DNA sequence comprising either: (a) a sequence set out in Figure 9; (b) any one of the subsequences A to N of Figure 9; (c) the sequence represented by bases 816 to 3204 of Figure 9; (d) a sequence set out in Figure 16; or (e) a DNA sequence hybridizable to (a), (b), (c) or (d); which sequence (a), (b), (c), (d) or (e) encodes a polypeptide which has PI3-kinase activity if expressed in insect cells or is capable of complexing with a p85 mammalian PI3-kinase subunit to produce such activity.

8. A DNA construct comprising a DNA sequence as defined in claim 7 under the control of a control sequence and in proper

reading frame in an expression vector, optionally the control sequence including a regulatable promoter.

9. Host cells which have been genetically altered by the incorporation therein of a construct as defined in claim 8 so as to permit expression of the encoded polypeptide, which host cells are optionally insect cells.

10. A method for the preparation of a polypeptide encoded by a DNA sequence as defined in claim 7, comprising cultivating host cells as defined in claim 9, which host cells are optionally insect cells.

11. A polypeptide obtainable by a method as defined in claim 10.

12. The use of a polypeptide as defined in any one of claims 1 to 5 or 11 to provide PI3-kinase activity, either directly or after complexing with a mammalian p85 subunit.

13. An enzymatically active complex of a peptide as defined in any one of claims 1 to 5 or 11 and a mammalian p85 subunit.

14. A method of prophylaxis or therapy which involves the encouragement or discouragement of cell proliferation by the action of an agonist or antagonist, respectively, for the PI3-kinase activity of a polypeptide as defined in any one of claims 1 to 5 or 11 or of an active complex including the same, wherein said cell proliferation is mediated through a cell surface receptor interactive with said activity.

15. A process for obtaining an agonist or antagonist for the PI3-kinase activity of a polypeptide as defined in any one of claims 1 to 5 or 11 or an active complex including the same, which process comprises screening candidate molecules for such activity using a polypeptide as defined in any one of claims 1 to 5 or 11.

16. A process for identifying molecules which bind specifically to a polypeptide as defined in any one of claims 1 to 5 or 11, which process comprises using such a polypeptide in a screening method for such binding.

17. A pharmaceutical or veterinary formulation comprising an agonist or antagonist for the PI3-kinase activity of a polypeptide as defined in any one of claims 1 to 5 or 11, or an active complex including the same, formulated for pharmaceutical or veterinary use, respectively, optionally together with an acceptable diluent, carrier or excipient and/or in unit dosage form.

18. The use of a polypeptide as defined in any one of claims 1 to 5 or 11, or of an active complex containing said polypeptide, or of an agonist or antagonist thereof, in affecting the level of stimulation of platelets or neutrophils or in regulating blood glucose levels.

19. A method of prophylaxis or therapy including the employment of a polypeptide as defined in any one of claims 1 to 5 or 11, or of an active complex containing said polypeptide, or an agonist or antagonist thereof, in affecting the level of stimulation of platelets or neutrophils or in regulating blood glucose levels.

20. The use of a polypeptide as defined in any of claims 1 to 5 or 11, or of an active complex containing said polypeptide, or of an agonist or antagonist thereof, in the manufacture of a medicament.

21. The use of a polypeptide as defined in any one of claims 1 to 5 or 11, or of an active complex containing said polypeptide, in the in vitro enzymatic production of 3-phosphorylated phosphoinositides.

22. A phosphorylated phosphoinositide obtained by the performance of the use as defined in claim 21.

23. A phosphotyrosine polypeptide incorporating tyrosine 751 of the human PDGF-β receptor having the capability of binding bovine brain PI3-kinase.

24. A human PI3-kinase p110 subunit sequence comprising amino acids 19 to 110 of human p110, or an amino terminal truncated or carboxy terminal truncated derivative thereof having less than 20 amino acids deleted from the amino terminal or carboxy terminal end, respectively, but which is capable of binding to a PI3-kinase p85 subunit.

25. A method of inhibiting p85 and p110 mammalian PI3-kinase subunit interaction, which comprises utilizing a molecule which blocks the binding domain located between amino acids 19 and 110 of human p110.

26. The use of a sequence or derivative as defined in claim 24 in screening for a therapeutic or prophylactic agent which operates by inhibiting interaction between p85 and p110 mammalian PI3-kinase subunits.

ABSTRACT

POLYPEPTIDES HAVING KINASE ACTIVITY, THEIR PREPARATION AND USE

This invention relates to new polypeptides which exhibit kinase activity or, more specifically, which show phosphoinositide (PI) 3-kinase activity. Such polypeptides are involved in pathways responsible for cellular growth and differentiation. An isolated polypeptide which possesses PI3-kinase activity when produced by recombinant production in insect cells is disclosed.

A

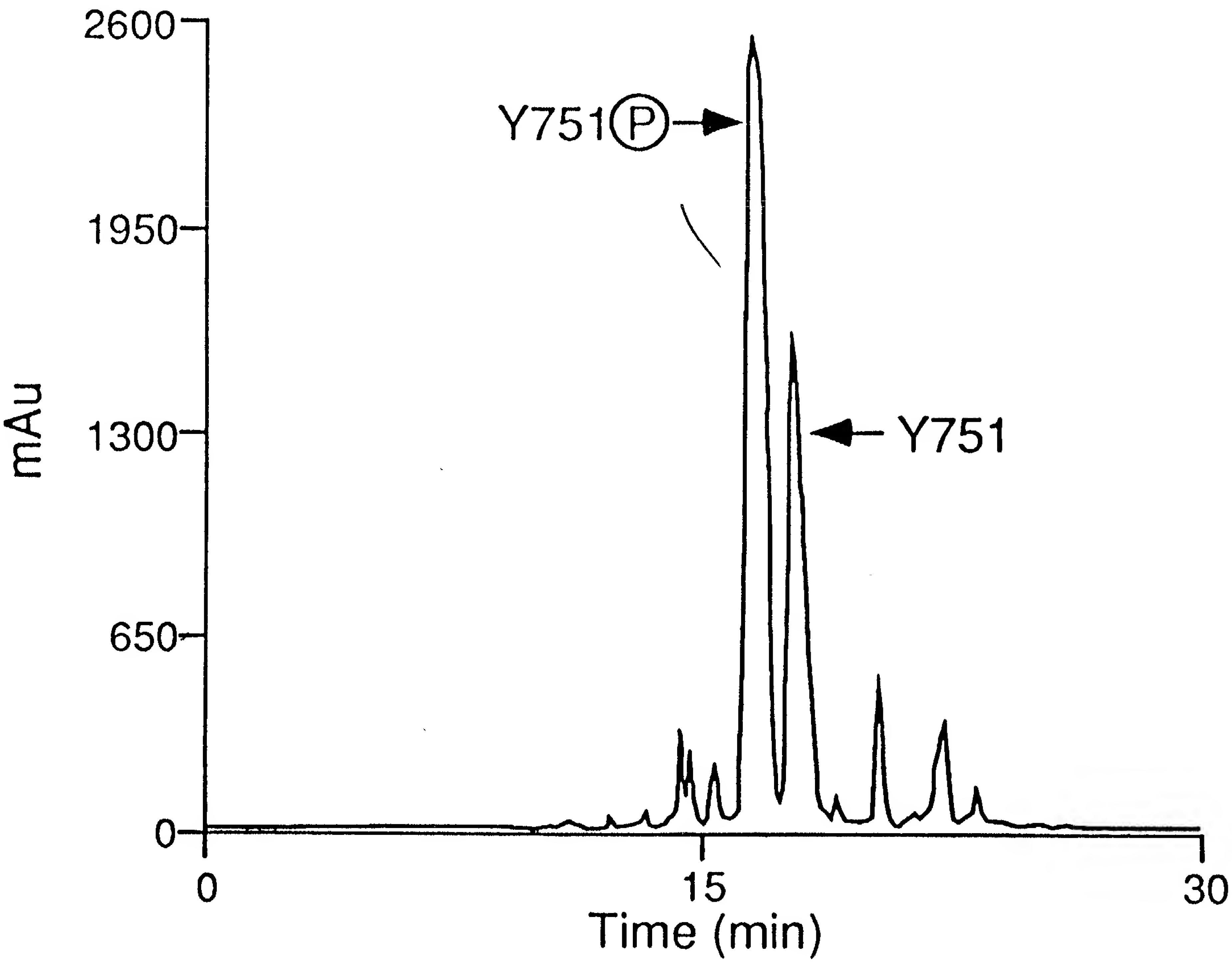


Figure 1

B

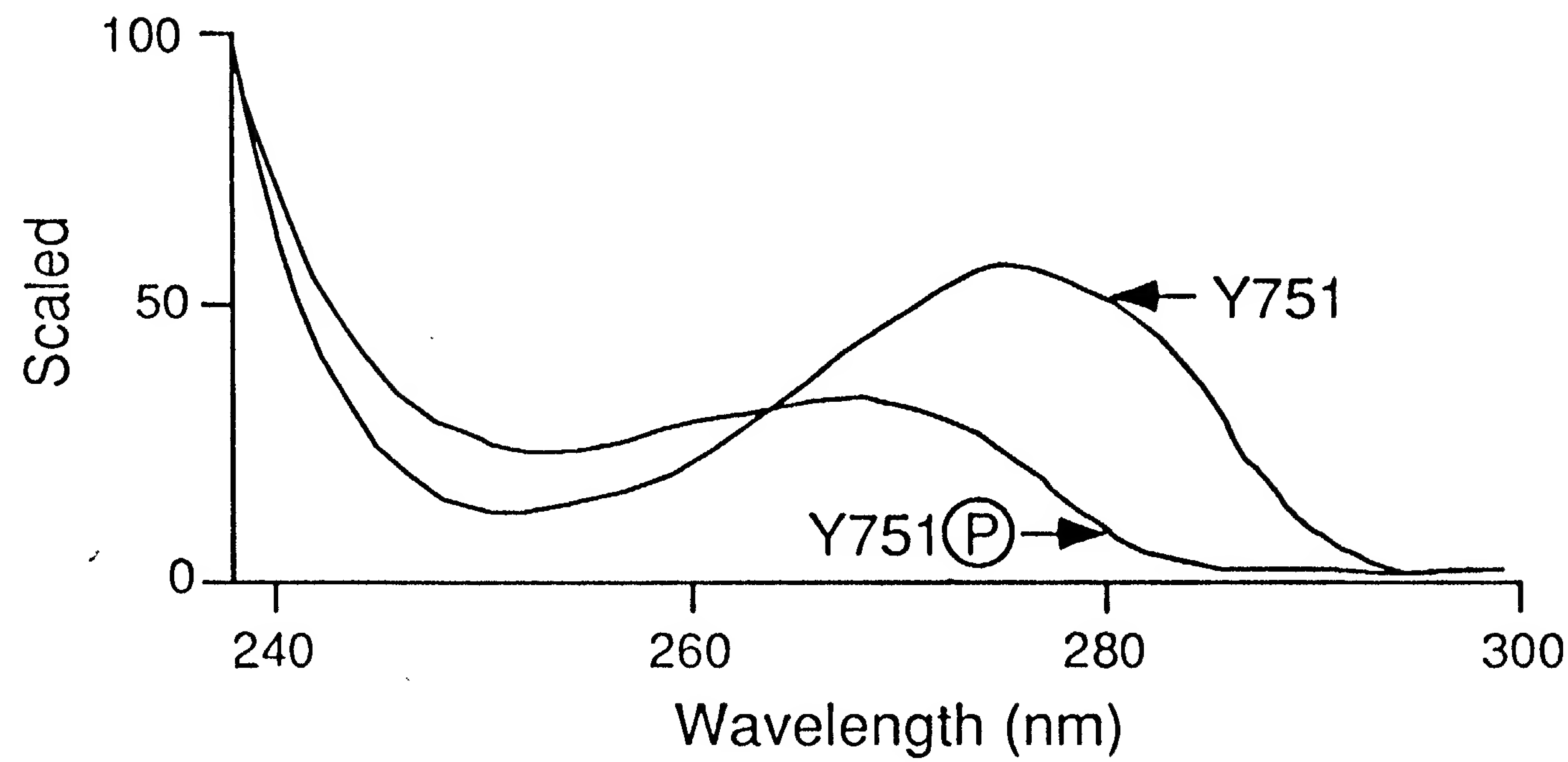


FIG 1 (contd)

C

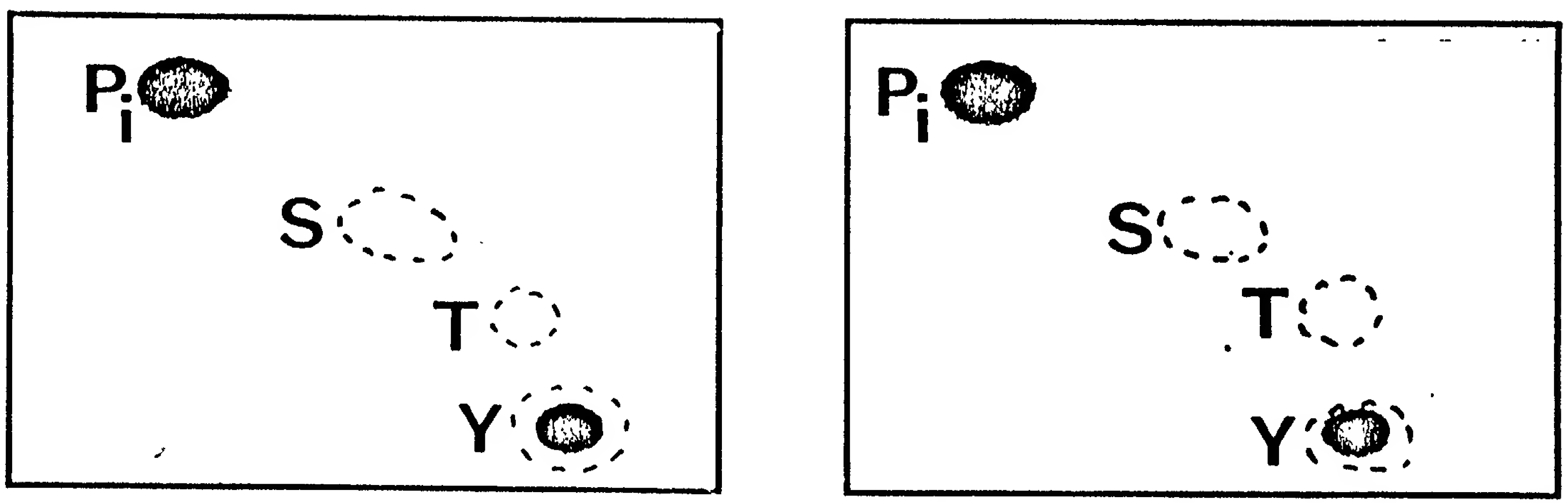


FIG 1 (contd)

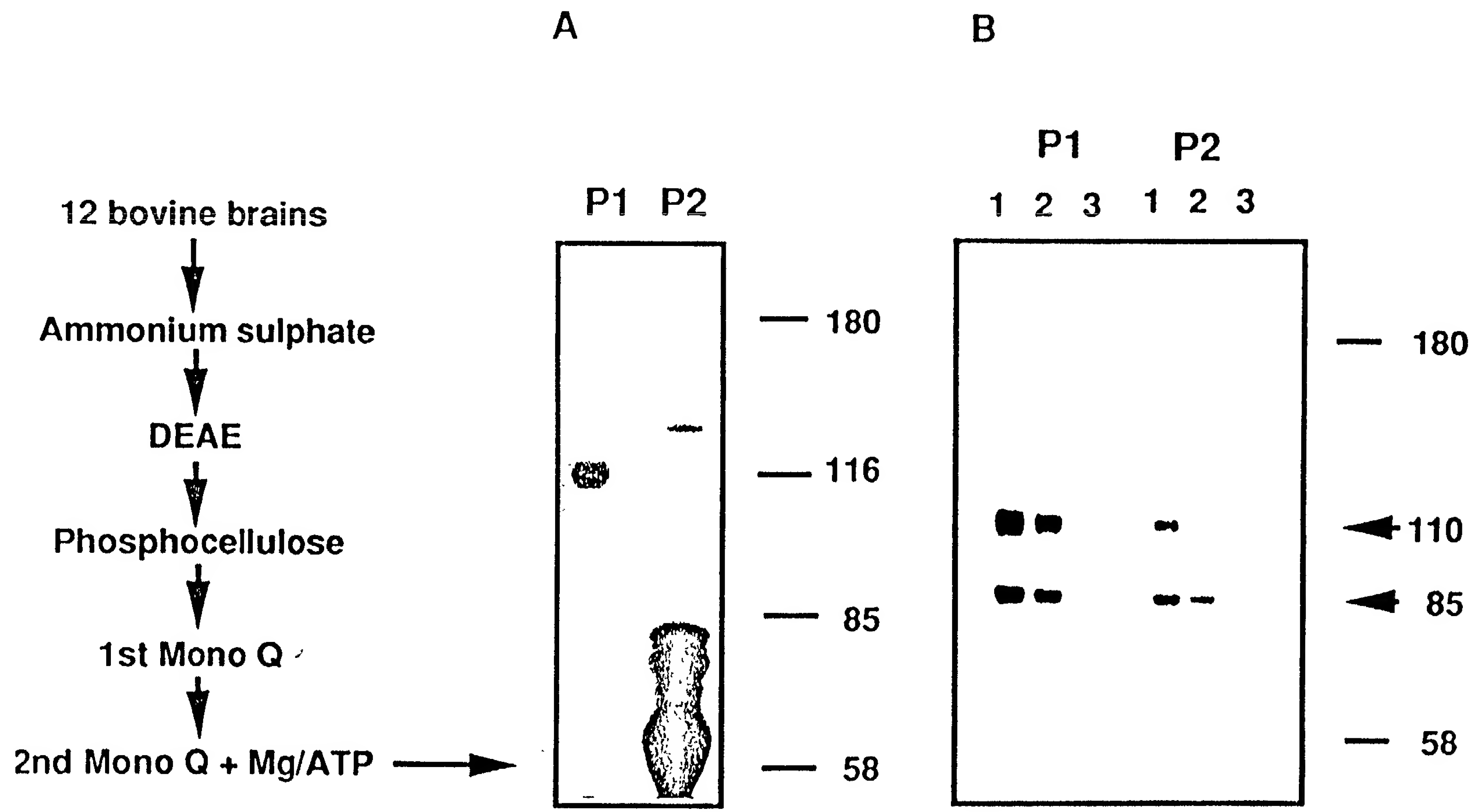

A
B
12 bovine brains
Ammonium sulphate
DEAE
Phosphocellulose
1st Mono Q
2nd Mono Q + Mg/ATP
P1 P2
180
116
85
58
P1
P2
1 2 3 1 2 3
180
110
85
58

Figure 2

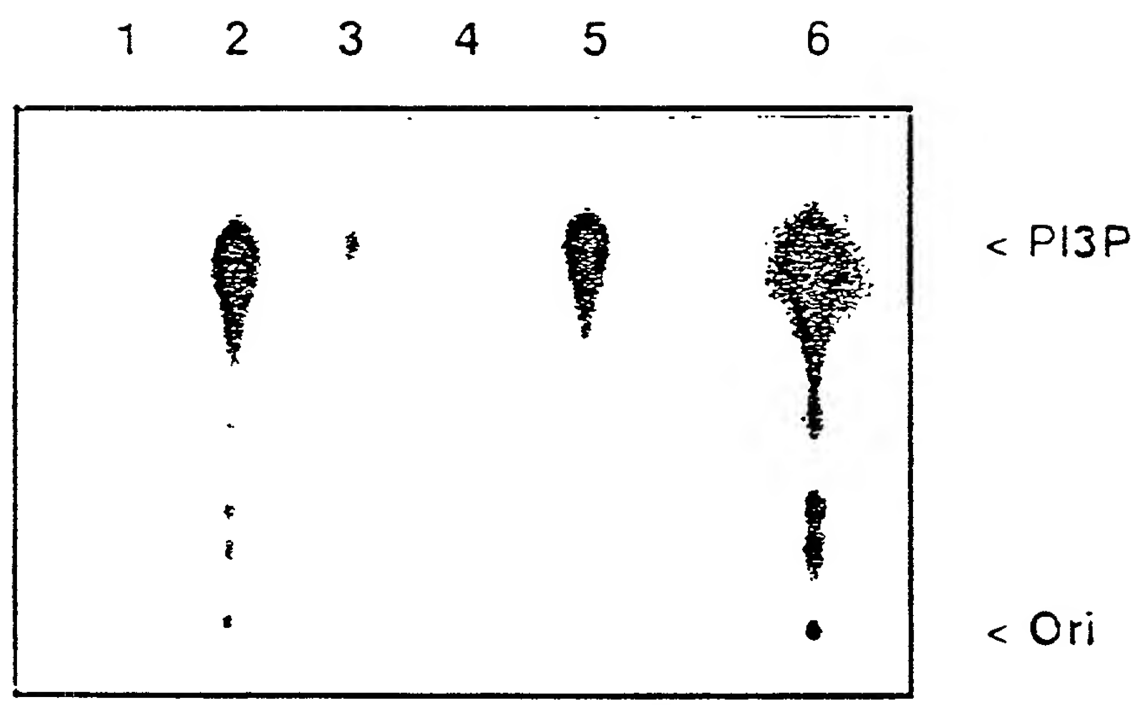
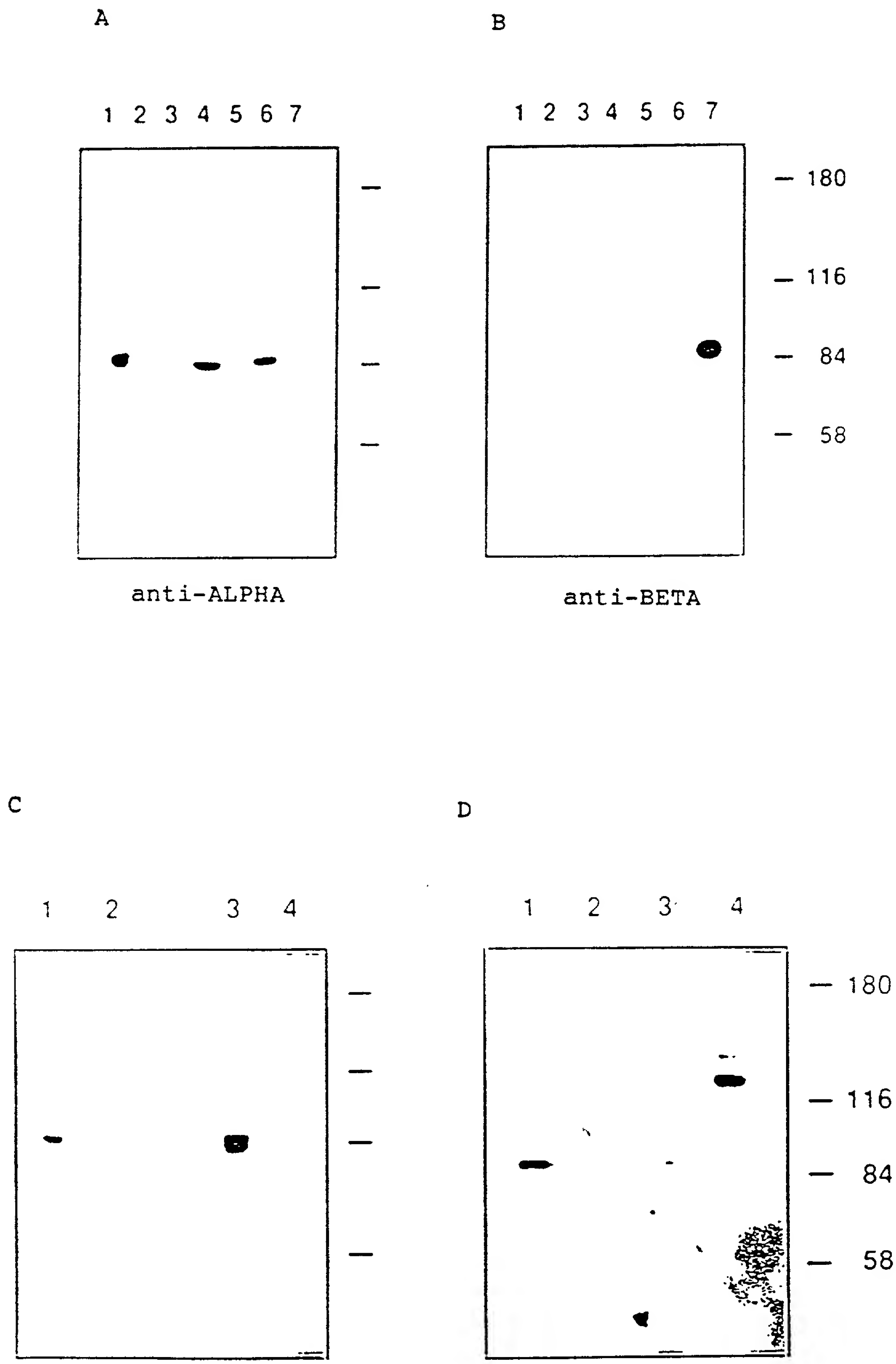


Figure 3

Figure 4


A
B
1 2 3 4 5 6 7
1 2 3 4 5 6 7
180
116
84
58
anti-ALPHA
anti-BETA
C
D
1 2 3 4
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180
116
84
58

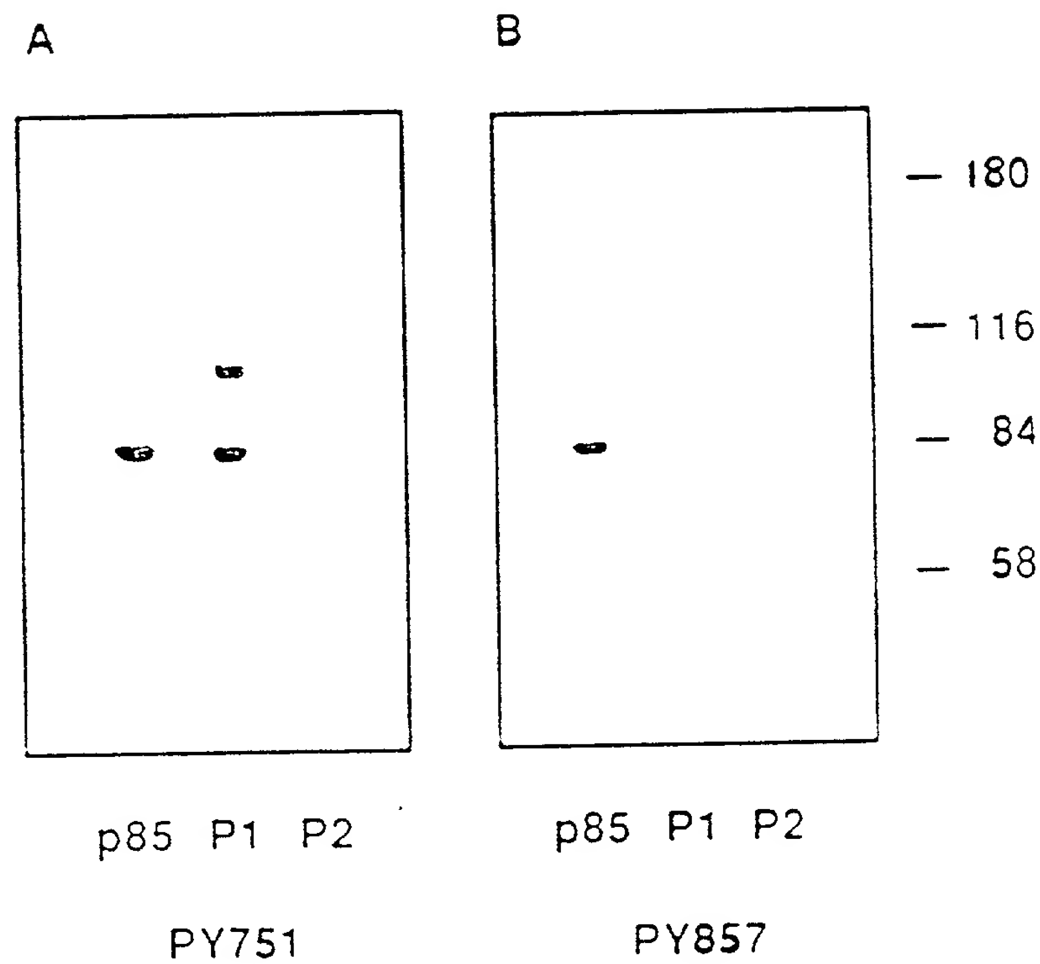
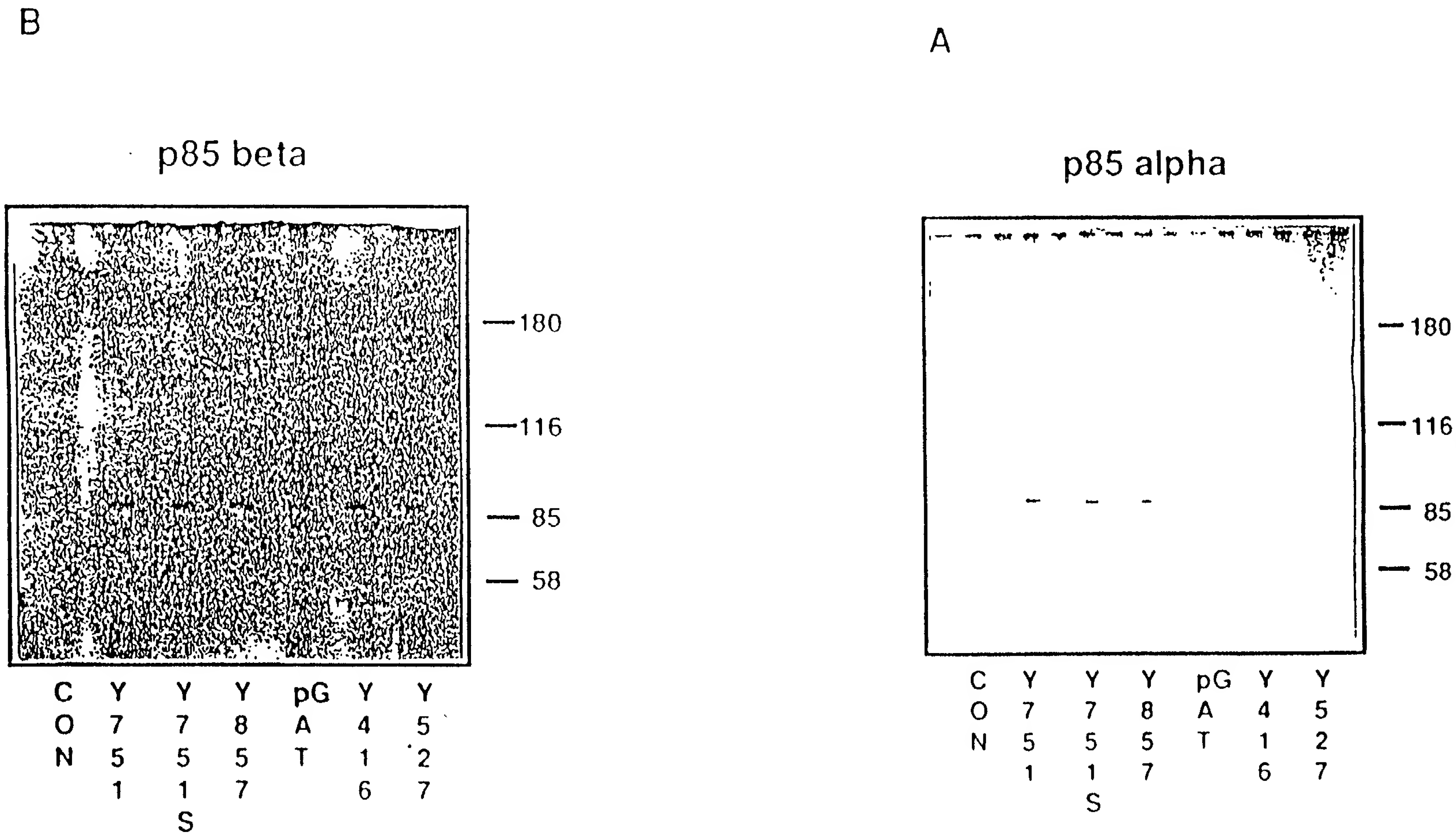


Figure 5


B
p85 beta
180
116
85
58
CON
Y751
Y751S
Y857
pGAT
Y416
Y527
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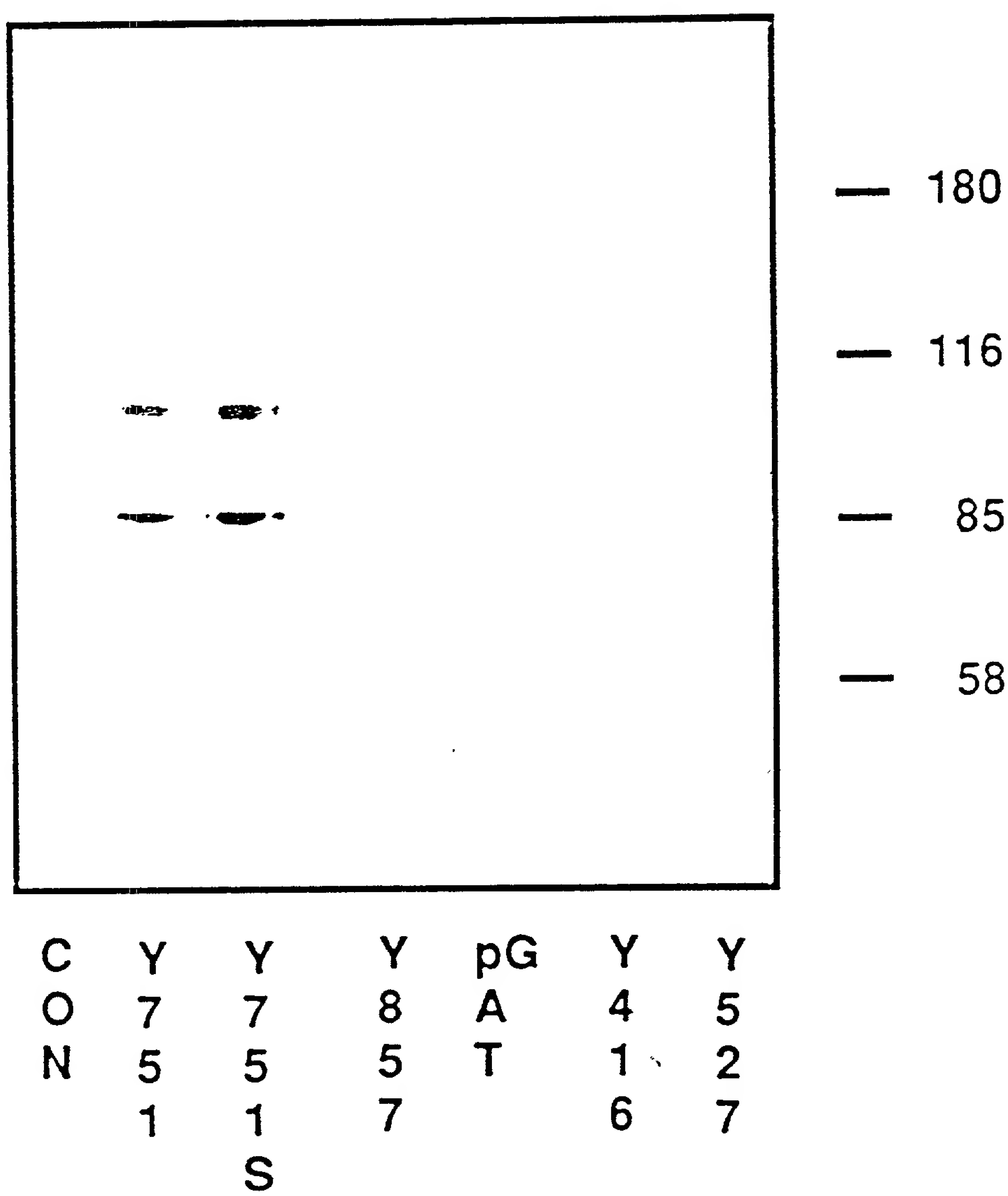
180
116
85
58
CON
Y751
Y751S
Y857
pGAT
Y416
Y527

Figure 6

Figure 7

A



B

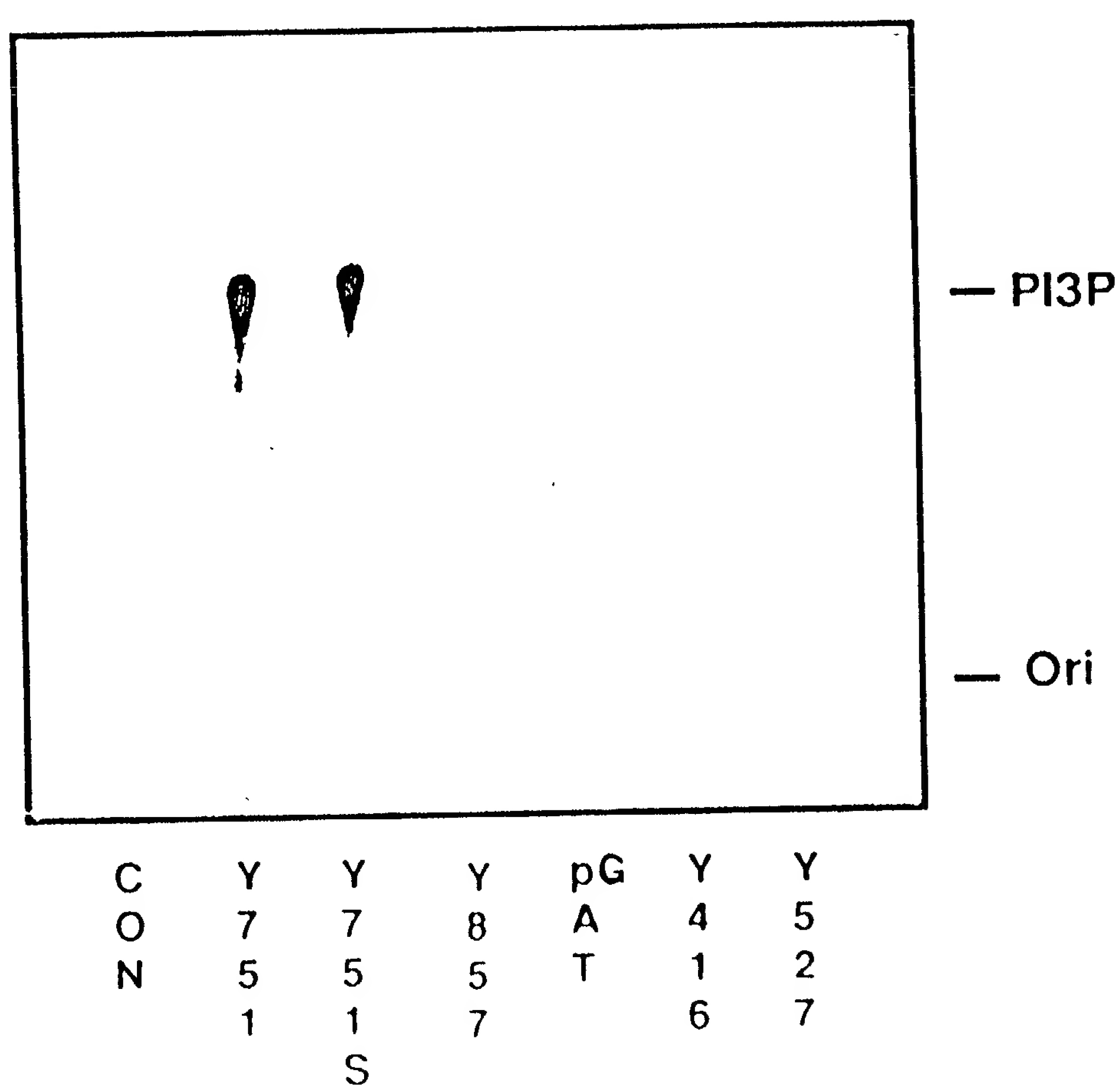
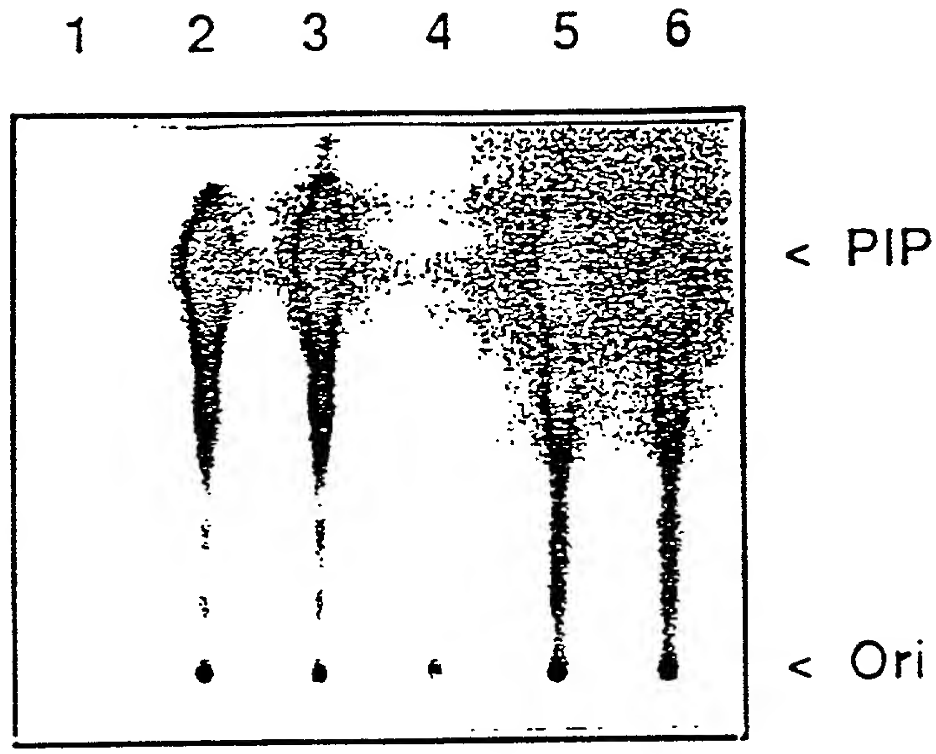


FIG 7 (contd)

Figure 8



751	D	M	S	K	D	E	S	V	D	Y	V	P	M	L	D	M	K
751.S				C	D	E	S	V	D	Y	V	P	M	L			
740				G	E	S	D	G	G	Y	M	D	M	S	K		
1313			E	F	C	P	D	P	L	Y	E	V	M	L	K		
Consensus					E	E	E	E	E	Y	M	P	M	X	X		
					D	D	D	D	D		V						

Figure 9

```
M  P  P  R  P  S  S  G  E  L  W  G  I  H  L  M      16
ATGCCTCCAAGACCATCATCAGGTGAACTGTGGGGCATCCACTTGATG   48

P  P  R  I  L  V  E  C  L  L  P  N  G  M  I  V      32
CCCCCAAGAATCCTAGTAGAATGTTTACTACCAAATGGGATGATAGTG   96

T  L  E  C  L  R  E  A  T  L  I  T  I  K  H  E      48
ACTTTAGAATGCCTCCGTGAGGCTACGTTAATAACGATAAAGCATGAA   144

L  F  K  E  A  R  K  Y  P  L  H  Q  L  L  Q  D      64
CTATTTAAAGAAGCAAGAAAATACCCTCTCCATCAACTTCTTCAAGAT   192

E  S  S  Y  I  F  V  S  V  T  Q  E  A  E  R  E      80
GAATCTTCTTACATTTTCGTAAGTGTTACCCAAGAAGCAGAAAGGGAA   240

E  F  F  D  E  T  R  R  L  C  D  L  R  L  F  Q      96
GAATTTTTTGATGAAACAAGACGACTTTGTGACCTTCGGCTTTTTCAA   288

P  F  L  K  V  I  E  P  V  G  N  R  E  E  K  I      112
CCCTTTTTAAAAGTAATTGAACCAGTAGGCAACCGTGAAGAAAAGATC   336

L  N  R  E  I  G  F  A  I  G  M  P  V  C  E  F      128
CTCAATCGAGAAATTGGTTTTGCTATCGGCATGCCAGTGTGTGAATTC   384

D  M  V  K  D  P  E  V  Q  D  F  R  R  N  I  L      144
GATATGGTTAAAGATCCAGAAGTACAGGACTTCCGAAGAAATATTCTC   432
```

FIG 9 (contd)

```
 N  V  C  K  E  A  V  D  L  R  D  L  N  S  P  H      160
AATGTTTGTAAAGAAGCTGTGGATCTTAGGATCTTAATTCACCTCAT      480
                       A
 S  R  A  M  Y  V  Y  P  P  N  V  E  S  S  P  E      176
AGTAGAGCAATGTATGTTTATCCTCCAAATGTAGAATCTTCACCAGAA     528

 L  P  K  H  I  Y  N  K  L  D  K  G  Q  I  I  V      192
CTGCCAAAGCACATATATAATAAATTGGATAAAGGGCAAATAATAGTG     576

 V  I  W  V  I  V  S  P  N  N  D  K  Q  K  Y  T      208
GTGATTTGGGTAATAGTTTCTCCAAATAATGACAAACAGAAGTATACT     624

 L  K  I  N  H  D  C  V  P  E  Q  V  I  A  E  A      224
CTGAAAATCAACCATGACTGTGTGCCAGAACAAGTAATTGCTGAAGCA     672

 I  R  K  K  T  R  S  M  L  L  S  S  E  Q  L  K      240
ATCAGGAAAAAAACTCGAAGTATGTTGCTATCATCTGAACAACTAAAA     720

 L  C  V  L  E  Y  Q  G  K  Y  I  L  K  V  C  G      256
CTCTGTGTTTTAGAATATCAGGGCAAGTATATTTTAAAAGTGTGTGGA     768

 C  D  E  Y  F  L  E  K  Y  P  L  S  Q  Y  K  Y      272
TGTGATGAATACTTCCTAGAAAAATATCCTCTGAGTCAGTATAAGTAT     816

 I  R  S  C  I  M  L  G  R  M  P  N  L  M  L  M      288
ATAAGAAGCTGTATAATGCTTGGGAGGATGCCCAATTTGATGCTGATG     864
```

FIG 9 (contd)

```
A  K  E  S  L  Y  S  Q  L  P  M  D  C  F  T  M          304
GCTAAAGAAAGCCTCTATTCTCAACTGCCAATGGACTGTTTTACAATG         912

P  S  Y  S  R  R  I  S  T  A  T  P  Y  M  N  G          320
CCATCATATTCCAGACGCATCTCCACAGCTACGCCATATATGAATGGA         960
                        B
E  T  S  T  K  S  L  W  V  I  N  S  A  L  R  I          336
GAAACATCTACAAAATCCCTTTGGGTTATAAATAGTGCACTCAGAATA        1008

K  I  L  C  A  T  Y  V  N  V  N  I  R  D  I  D          352
AAAATTCTTTGTGCAACCTATGTGAATGTAAATATTCGAGACATTGAC        1056

K  I  Y  V  R  T  G  I  Y  H  G  G  E  P  L  C          368
AAGATTTATGTTCGAACAGGTATCTACCATGGAGGAGAACCCTTATGT        1104

D  N  V  N  T  Q  R  V  P  C  S  N  P  R  W  N          384
GATAATGTGAACACTCAAAGAGTACCTTGTTCCAATCCCAGGTGGAAT        1152

E  W  L  N  Y  D  I  Y  I  P  D  L  P  R  A  A          400
GAATGGCTGAATTACGATATATACATTCCTGATCTTCCTCGTGCTGCT        1200

R  L  C  L  S  I  C  S  V  K  G  R  K  G  A  K          416
CGACTTTGCCTTTCCATTTGTTCTGTTAAAGGCCGAAAGGGTGCTAAA        1248

E  E  H  C  P  L  A  W  G  N  I  N  L  F  D  Y          432
GAGGAACACTGTCCATTGGCCTGGGAAATATAAACTTGTTTGATTAC         1296
```

FIG. 9 (contd)

```
T  D  T  L  V  S  G  K  M  A  L  N  L  W  P  V     448
ACAGATACTCTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTA  1344
                    C
P  H  G  L  E  D  L  L  N  P  I  G  V  T  G  S     464
CCTCATGGACTAGAAGATTTGCTGAACCCTATTGGTGTTACTGGATCA  1392

N  P  N  K  E  T  P  C  L  E  L  E  F  D  W  F     480
AATCCAAATAAAGAAACTCCATGTTTAGAGTTGGAGTTTGACTGGTTC  1440

S  S  V  V  K  F  P  D  M  S  V  I  E  E  H  A     496
AGCAGTGTGGTAAAGTTTCCAGATATGTCAGTGATTGAAGAGCATGCC  1488

N  W  S  V  S  R  E  A  G  F  S  Y  S  H  A  G     512
AATTGGTCTGTATCCCGTGAAGCAGGATTTAGTTATTCCCATGCAGGA  1536

L  S  N  R  L  A  R  D  N  E  L  R  E  N  D  K     528
CTGAGTAACAGACTAGCTAGAGACAATGAATTAAGAGAAAATGATAAA  1584

E  Q  L  R  A  I  C  T  R  D  P  L  S  E  I  T     544
GAACAGCTCCGAGCAATTTGTACACGAGATCCTCTATCTGAAATCACT  1632

E  Q  E  K  D  F  L  W  S  H  R  H  Y  C  V  T     560
GAGCAAGAGAAAGATTTTCTGTGGAGCCACAGACACTATTGTGTAACT  1680

I  P  E  I  L  P  K  L  L  L  S  V  K  W  N  S     576
ATCCCCGAAATTCTACCCAAATTGCTTCTGTCTGTTAAATGGAACTCT  1728
```

FIG 9 (contd)

```
 R  D  E  V  A  Q  M  Y  C  L  V  K  D  W  P  P       592
AGAGATGAAGTAGCTCAGATGTACTGCTTGGTAAAAGATTGGCCTCCA    1776

 I  K  P  E  Q  A  M  E  L  L  D  C  N  Y  P  D       608
ATCAAGCCTGAACAGGCTATGGAGCTTCTGGACTGCAATTACCCAGAT    1824

 P  M  V  R  G  F  A  V  R  C  L  E  K  Y  L  T       624
CCTATGGTTCGAGGTTTTGCTGTTCGGTGCTTAGAAAAATATTTAACA    1872
                      D
 D  D  K  L  S  Q  Y  L  I  Q  L  V  Q  V  L  K       640
GATGACAAACTTTCTCAGTACCTAATTCAGCTAGTACAGGTACTAAAA    1920

 Y  E  Q  Y  L  D  N  L  L  V  R  F  L  L  K  K       656
TATGAACAGTATTTGGATAACCTGCTTGTGAGATTTTTACTCAAAAAA    1968
                      E
 A  L  T  N  Q  R  I  G  H  F  F  F  W  H  L  K       672
GCGTTAACTAATCAAAGGATCGGTCACTTTTTCTTTTGGCATTTAAAA    2016
                                    F
 S  E  M  H  N  K  T  V  S  Q  R  F  G  L  L  L       688
TCTGAGATGCACAATAAAACAGTTAGTCAGAGGTTTGGCCTGCTTTTG    2064

 E  S  Y  C  R  A  C  G  M  Y  L  K  H  L  N  R       704
GAGTCCTATTGCCGTGCATGTGGGATGTATCTGAAGCACCTTAATAGG    2112
                                 G
 Q  V  E  A  M  E  K  L  I  N  L  T  D  I  L  K       720
CAAGTTGAGGCTATGGAAAAGCTCATTAACTTGACTGACATTCTCAAA    2160
```

FIG 9 (contd)

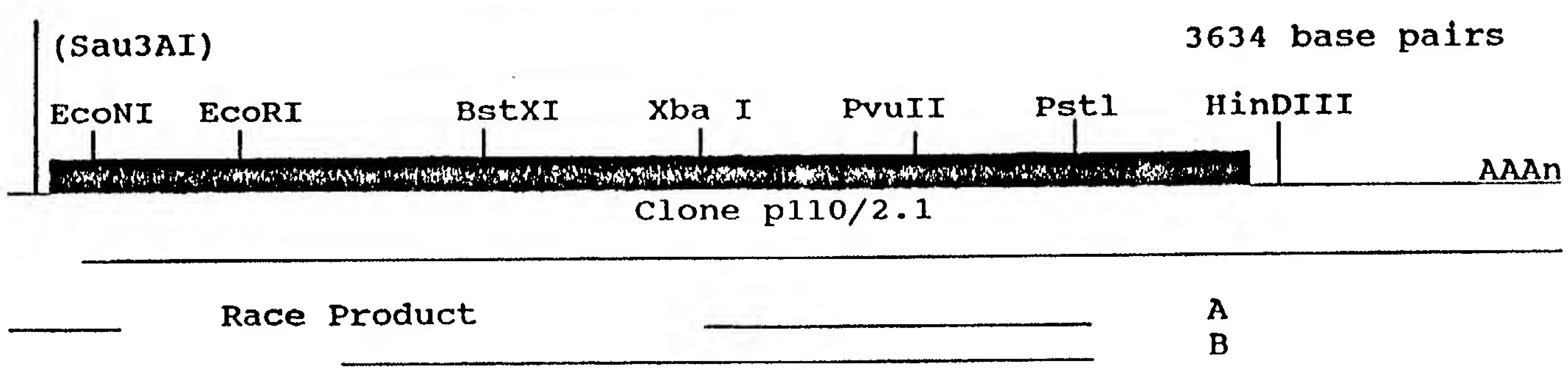
```
Q   E   K   K   D   E   T   Q   K   V   Q   M   K   F   L   V     736
CAAGAGAAGAAGGATGAAACACAAAAGGTACAGATGAAGTTTTTAGTT               2208

E   Q   M   R   R   P   D   F   M   D   A   L   Q   G   F   L     752
GAGCAAATGCGGCGACCAGATTTCATGGATGCTCTCCAGGGCTTTCTG               2256

S   P   L   N   P   A   H   Q   L   G   N   L   R   L   E   E     768
TCTCCTCTAAACCCTGCTCATCAGCTGGGAAATCTCAGGCTTGAAGAG               2304

C   R   I   M   S   S   A   K   R   P   L   W   L   N   W   E     784
TGTCGAATTATGTCTTCTGCAAAAAGGCCACTGTGGTTGAATTGGGAG               2352

N   P   D   I   M   S   E   L   L   F   Q   N   N   E   I   I     800
AACCCAGACATCATGTCAGAATTACTCTTTCAGAACAATGAGATCATC               2400

F   K   N   G   D   D   L   R   Q   D   M   L   T   L   Q   I     816
TTTAAAAATGGGGATGATTTACGGCAAGATATGCTAACCCTTCAGATT               2448

I   R   I   M   E   N   I   W   Q   N   Q   G   L   D   L   R     832
ATTCGCATTATGGAAAATATCTGGCAAAATCAAGGTCTTGATCTTCGA               2496

M   L   P   Y   G   C   L   S   I   G   D   C   V   G   L   I     848
ATGTTACCTTATGGATGTCTGTCAATCGGTGACTGTGTGGGACTTATC               2544

E   V   V   R   N   S   H   T   I   M   Q   I   Q   C   K   G     864
GAGGTGGTGAGAAATTCTCACACTATAATGCAGATTCAGTGTAAAGGA               2592
```

FIG 9 (contd)

```
                                    H
G  L  K  G  A  L  Q  F  N  S  H  T  L  H  Q  W       880
GGCCTGAAAGGTGCACTGCAGTTTAACAGCCACACACTCCATCAGTGG    2640

L  K  D  K  N  K  G  E  I  Y  D  A  A  I  D  L       896
CTCAAAGACAAGAACAAGGGGGAAATATATGATGCGGCCATCGATTTG    2688
            I
F  T  R  S  C  A  G  Y  C  V  A  T  F  I  L  G       912
TTTACACGATCATGTGCTGGATATTGTGTTGCCACCTTCATTTTGGGA    2736

I  G  D  R  H  N  S  N  I  M  V  K  D  D  G  Q       928
ATTGGAGATCGTCACAATAGTAATATCATGGTTAAAGATGATGGACAA    2784
                  J
L  F  H  I  D  F  G  H  F  L  D  H  K  K  K  K       944
CTGTTTCATATAGATTTTGGACACTTTTTGGATCACAAGAAGAAAAAA    2832
                                 K
F  G  Y  K  R  E  R  V  P  F  V  L  T  Q  D  F       960
TTTGGTTATAAACGAGAGCGCGTGCCGTTTGTTTTGACACAAGATTTC    2880

L  I  V  I  S  K  G  A  Q  E  C  T  K  T  R  E       976
TTAATAGTGATTAGTAAAGGAGCCCAAGAATGCACAAAGACAAGAGAA    2928

F  E  R  F  Q  E  M  C  Y  K  A  Y  L  A  I  R       992
TTTGAGAGGTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTATTCGG    2976
```

FIG 9 (contd)

```
                        L
Q  H  A  N  L  F  I  N  L  F  S  M  M  L  G  S      1008
CAGCATGCCAATCTCTTCATAAATCTTTTCTCAATGATGCTTGGCTCT    3024

G  M  P  E  L  Q  S  F  D  D  I  A  Y  I  R  K      1024
GGAATGCCAGAACTGCAATCTTTTGATGATATTGCATACATTCGAAAG    3072
                                 M
T  L  A  L  D  K  T  E  Q  E  A  L  E  Y  F  M      1040
ACCCTAGCTTTAGATAAAACTGAGCAAGAGGCTTTGGAGTATTTCATG    3120

K  Q  M  N  D  A  H  H  G  G  W  T  T  K  M  D      1056
AAACAAATGAATGATGCACACCATGGTGGCTGGACAACAAAAATGGAT    3168
         N
W  I  F  H  T  I  K  Q  H  A  L  N  *   1069
TGGATCTTCCACACAATTAAGCAGCATGCTTTGAACTGA   3207
```



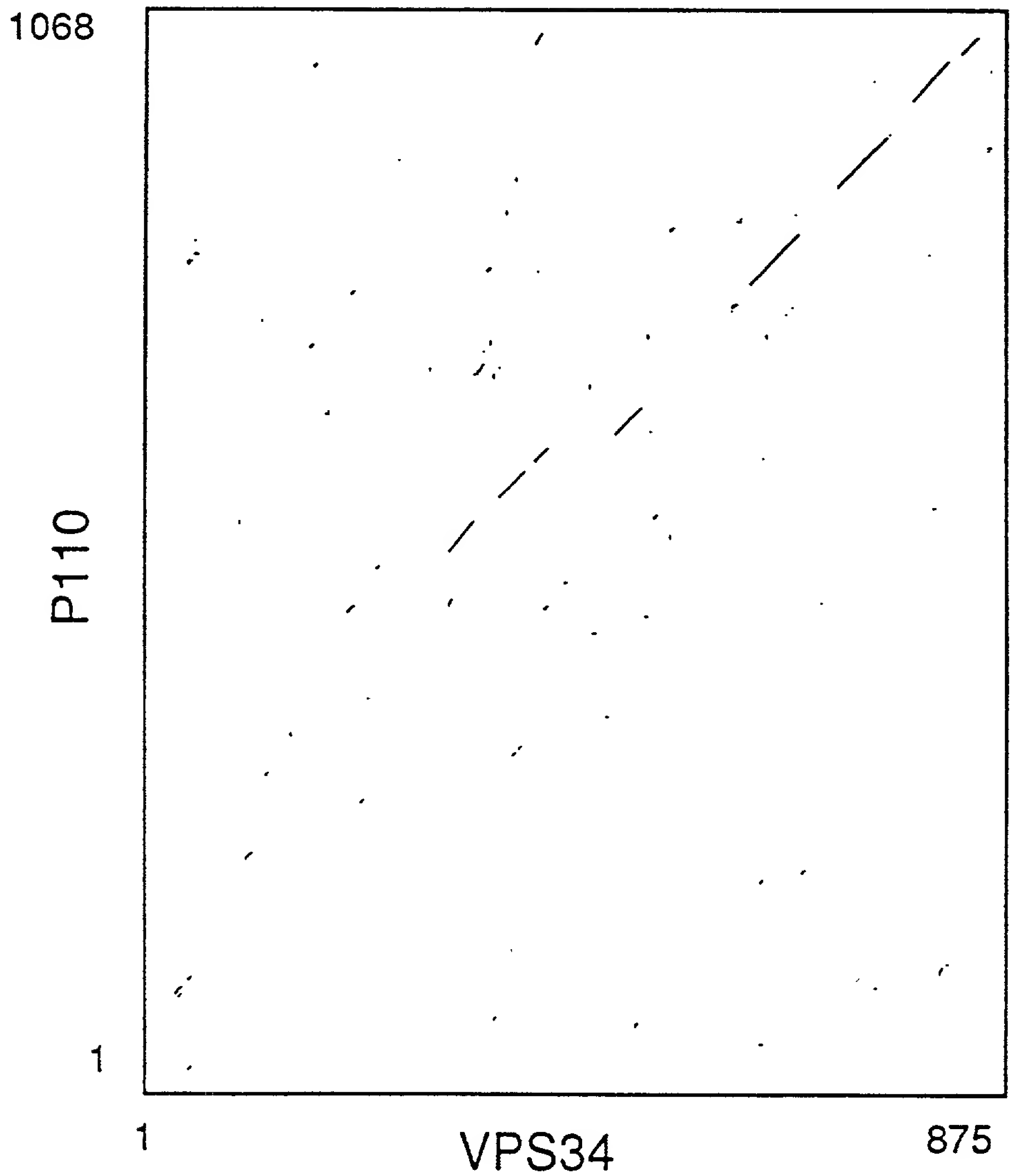


Figure 10 A

Figure 10 B

```
                    .         .         .         .         .
P110         VCEEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPN 170
              ..| : .| :|. :: .| .:  . . |:. ..  ...: :...|
VPS34        NITFCVSQDLDVP.LKVKIKSLEGHKPLLKPSQKILNPELMLIGSN 49

                    .         .         .         .         .
    171 VESSPEL..PKHIYNKLDKGQIIVVIWVIVSPNNDKQKYTLKINHDCVPE 218
        | .|.:|  . ::::|  . .:.:.|:.   | .:.:..:...:
     50 VFPSSDLIVSLQVFDKERNRNLTLPIYTPYIPFRNSRTWDYWL....... 92

                    .         .         .         .         .
    219 QVIAEAIRKKTRSMLLSSEQLKLCVLEYQGKYILKVCGCDEYFLEKYPLS 268
             .:. :.: : :|| :|::.::||.|
     93 .....TLPIRIKQLTFSS.HLRIILWEYNG.................... 116

                    .         .         .         .         .
    269 QYKYIRSCIMLGRMPNLMLMAKESLYSQLPMDCFTMPSYSRRISTATPYM 318
                                                   |...|::
    117 ...........................................SKQIPFF 123

                    .         .         .         .         .
    319 NGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGIYHGGEPLC 368
        | |||. : : ::.|:               |::::: .| ::.. .|.:.
    124 NLETSI..FNLKDCTLK..............RGFESLKFRYDVIDHCEVVT 158

                    .         .         .         .         .
    369 DNVNTQRVPCSNPRWNEWLNYDIYIPDLPRAARLC.LSICSVKGRKGAKE 417
        || :              .| || ..: .::.| ::|  :.|:.:: .::.:.
    159 DNKD...........QENLN.KYFQGEFTRLPWLDEITISKLRKQRENRT 196
```

Fig 10 B (contd)

```
                 .         .         .         .         .
418  .EHCPLAWG.NINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGS.  464
      .:..:.:. :: :::.. .::. .:  .    :|     .| |  |:...
197  WPQGTFVLNLEFPMLELPVVFIEREIMNTQMNIP....TLKNNPGLSTDL  242

                 .         .         .         .         .
465  .NPNKETPCLELEF.DWFSSVVKFPDMSVIEEHANWSVSREAGFSYSHAG  512
      :||::.| :.:.: |.: |.:|| |     .:::| ..  |   ...:|:
243  REPNRNDPQIKISLGDKYHSTLKFYD....PDQPNNDPIEEKYRRLERAS  288

                 .         .         .         .         .
513  LSNRLARDNELRENDKEQLRAICTRDPLSEITEQEKDFLWSHRHYCVTIP  562
      ...|.:: .   ..:: |. |.. .| ..:|.:||: :|..|.| :. .
289  KNANLDKQVKPDIKKRDYLNKIINYPPGTKLTAHEKGSIWKYRYYLMNNK  338

                 .         .         .         .         .
563  EILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQAMELLDCNYPDPMVR  612
     . |.||| |..:.. .| .::. |:..|:.|. ::|:|||::.:.:  ||
339  KALTKLLQSTNLREESERVEVLELMDSWAEIDIDDALELLGSTFKNLSVR  388

                 .         .         .         .         .
613  GFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQY..................  644
     ::||. |.|  .|..|. ||:|||:.: :|..
389  SYAVNRLKK.ASDKELELYLLQLVEAVCFENLSTFSDKSNSEFTIVDAVS  437

                 .         .         .         .         .
645  ....................................LDNLLVRFLLKK  656
                                         : . |. ||:::
438  SQKLSGDSMLLSTSHANQKLLKSISSESETSGTESLPIVISPLAEFLIRR  487
```

Fig. 10 B (contd)

```
                 .         .         .         .         .
657 ALTNQRIGHFFFWHLKSEMHNKTVSQRFGLLLESY.CRACGMYLKHLNRQ 705
    ||.|.|:| ||:|.||||  .:|.     :: :|.|: :|   .  . || |
488 ALVNPRLGSFFYWYLKSESEDKPY...LDQILSSFWSRLDKKSRNILNDQ 534

                 .         .         .         .         .
706 VEAMEKLINLTDILKQEKKDETQKVQMKF.LVEQMRRPDFMDALQGFLSP 754
    |  :: | : .:.:|. |.....|::: . |:| . ||  :  :..:  |
535 VRLINVLRECCETIKRLKDTTAKKMELLVHLLETKVRP..LVKVRPIALP 582

                 .         .         .         .         .
755 LNPAHQLGNLRLEECRIMSSAKRPLWLNWENPDIMSELLFQNNEIIFKNG 804
    |:|.  :.:: |.::::.|. .||.:.:...     | |  .::|| |
583 LDPDVLICDVCPETSKVFKSSLSPLKITFKTT......LNQPYHLMFKVG 626

                 .         .         .       *.         .
805 DDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLSIGDCVGLIEVVRNS 854
    |||||| |.:|||.:|::::.|:.:||:: || .|..|.  | ||.:.|
627 DDLRQDQLVVQIISLMNELLKNENVDLKLTPYKILATGPQEGAIEFIPN. 675

               *.         .         .         .         .
855 HTIMQIQCK.GGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLFTRSCAG 903
    .|:  | :|  |: | |.:      :: .:... :: : .:| |.:||||
676 DTLASILSKYHGILGYLKL......HYPDENATLGVQGWVLDNFVKSCAG 719

                 .  *    *  .          ***          .         .
904 YCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVP 953
    ||| |:|||:|||| .|::|..||::|| |||.:|:::..|.|      |
720 YCVITYILGVGDRHLDNLLVTPDGHFFHADFGYILGQDPKPF.......P 762
```

Fig 10 B (contd)

```
               .         .         .         .         .
954  FVLTQDFLIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFS 1003
      ::. .  |: . |:.|:.   ::::|.. |: ||  :|.:|.|::|||.
763  PLMKLPPQIIEAFGGAESS...NYDKFRSYCFVAYSILRRNAGLIILNLFE 809

               .         .         .         .         .
1004 MMLGSGMPE..LQSFDDIAYIRKTLALDKTEQEALEYFMKQMNDAHHGGW 1051
     :| .|.:|:  ::. :.|  :|. :.|:..|::|  .| . :||. :: :
810  LMKTSNIPDIRIDPNGAILRVRERFNLNMSEEDATVHFQNLINDSVNALL 859

               .
1052 TTKMDWIFHTIKQH 1065
     .. :|  :|.: |.
860  PIVIDH.LHNLAQY 872
```

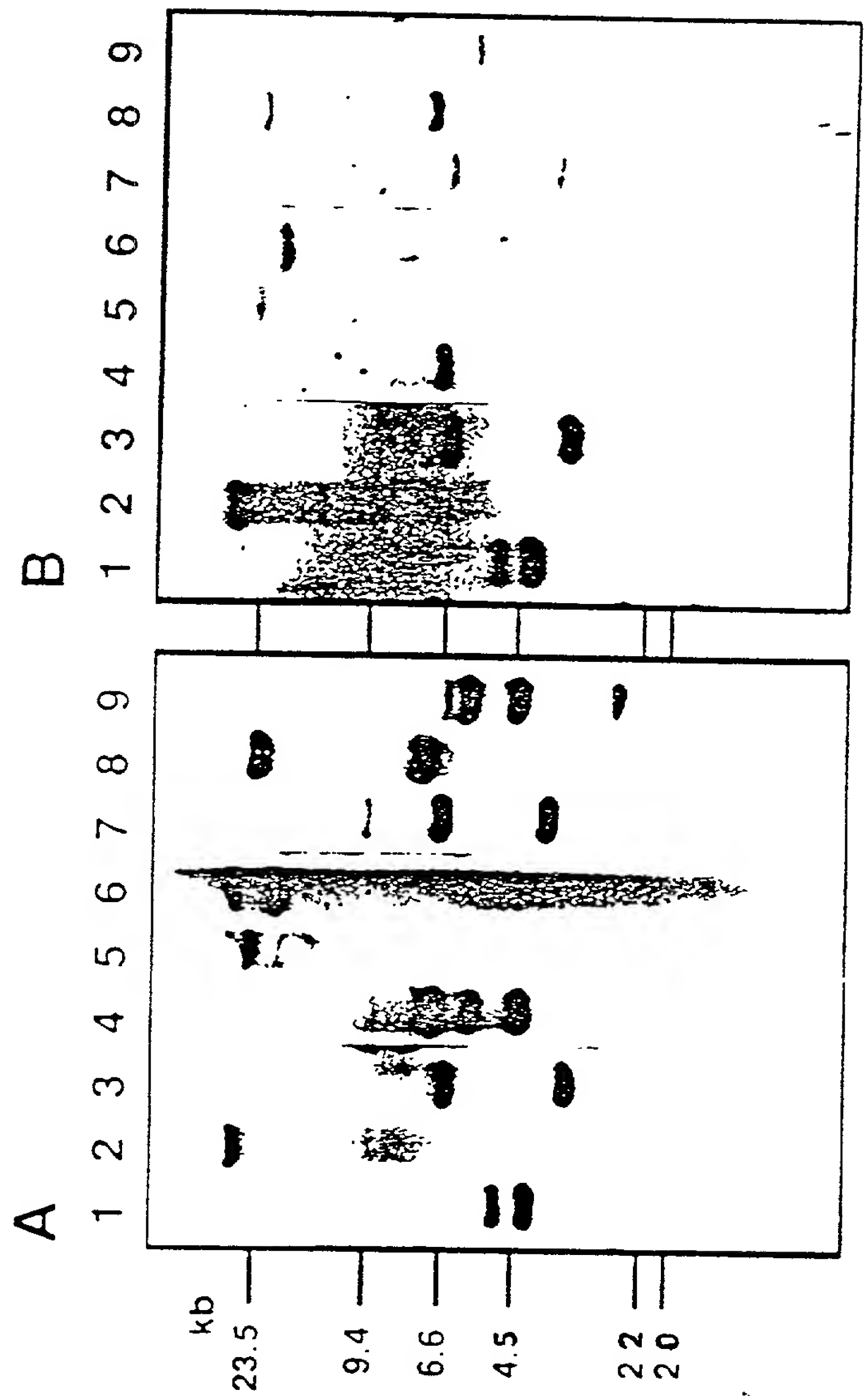


Figure 11

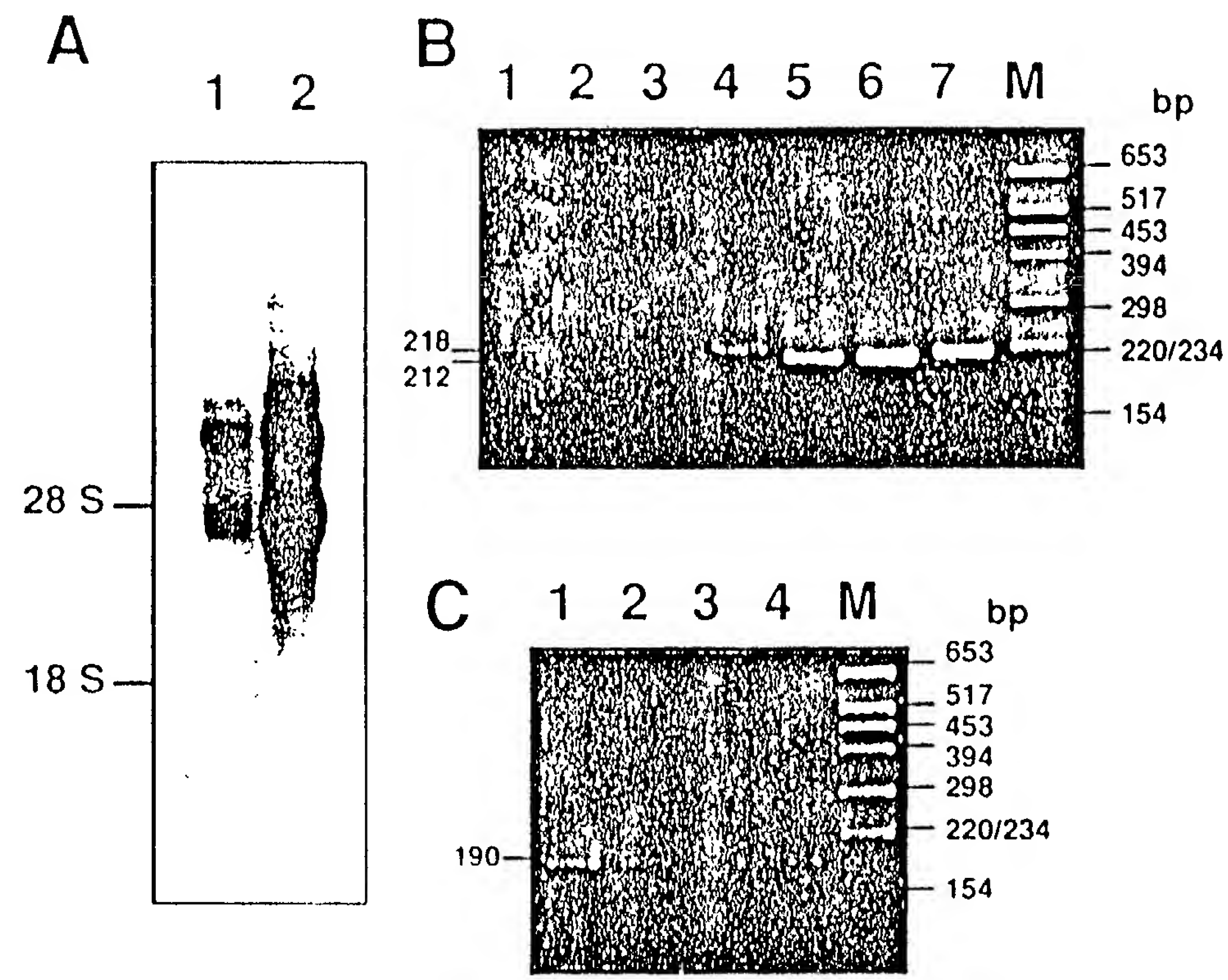

A
1 2
28 S
18 S
B
1 2 3 4 5 6 7 M
bp
653
517
453
394
298
220/234
154
218
212
C
1 2 3 4 M
bp
653
517
453
394
298
220/234
154
190

Figure 12

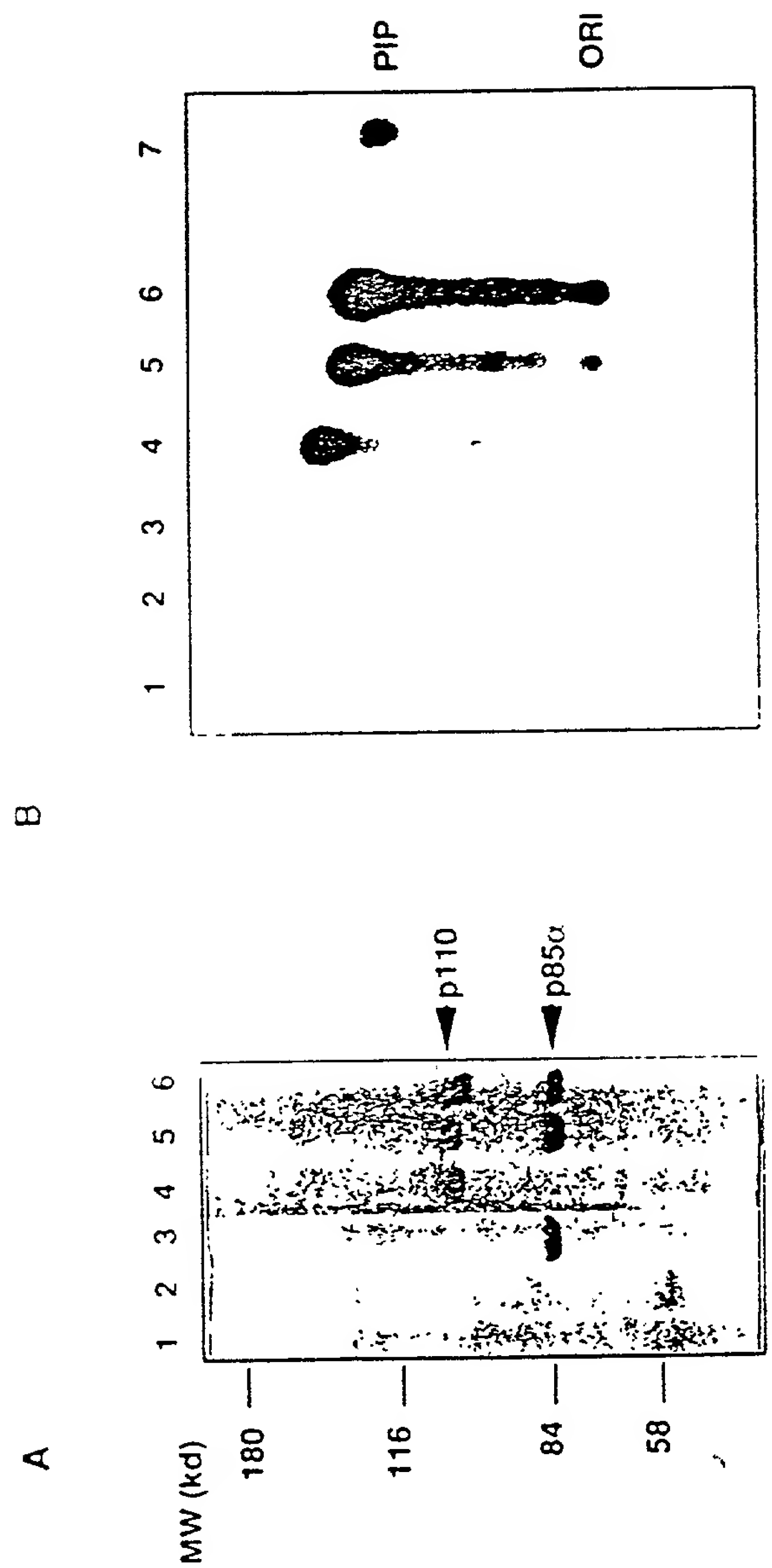


Figure 13

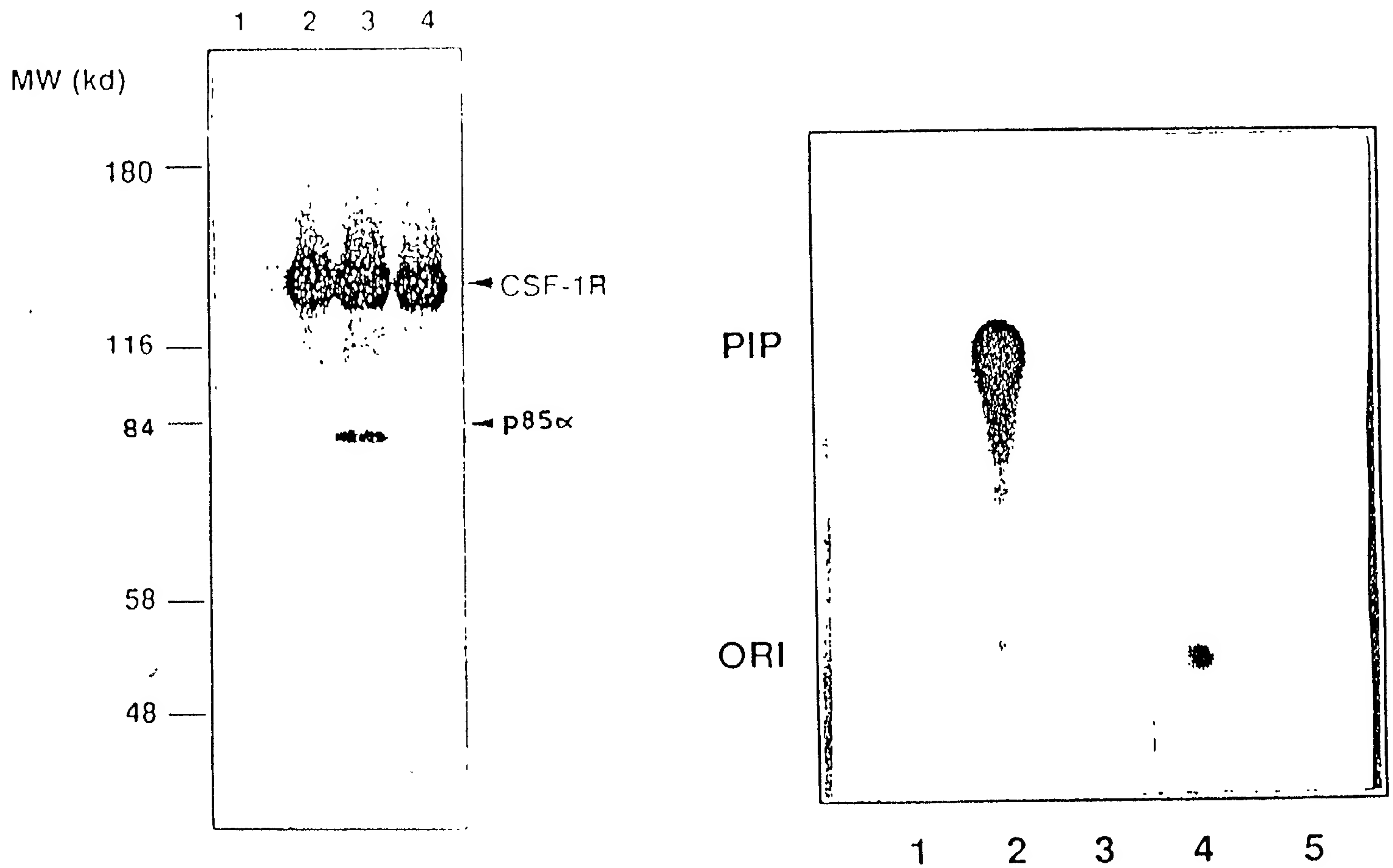
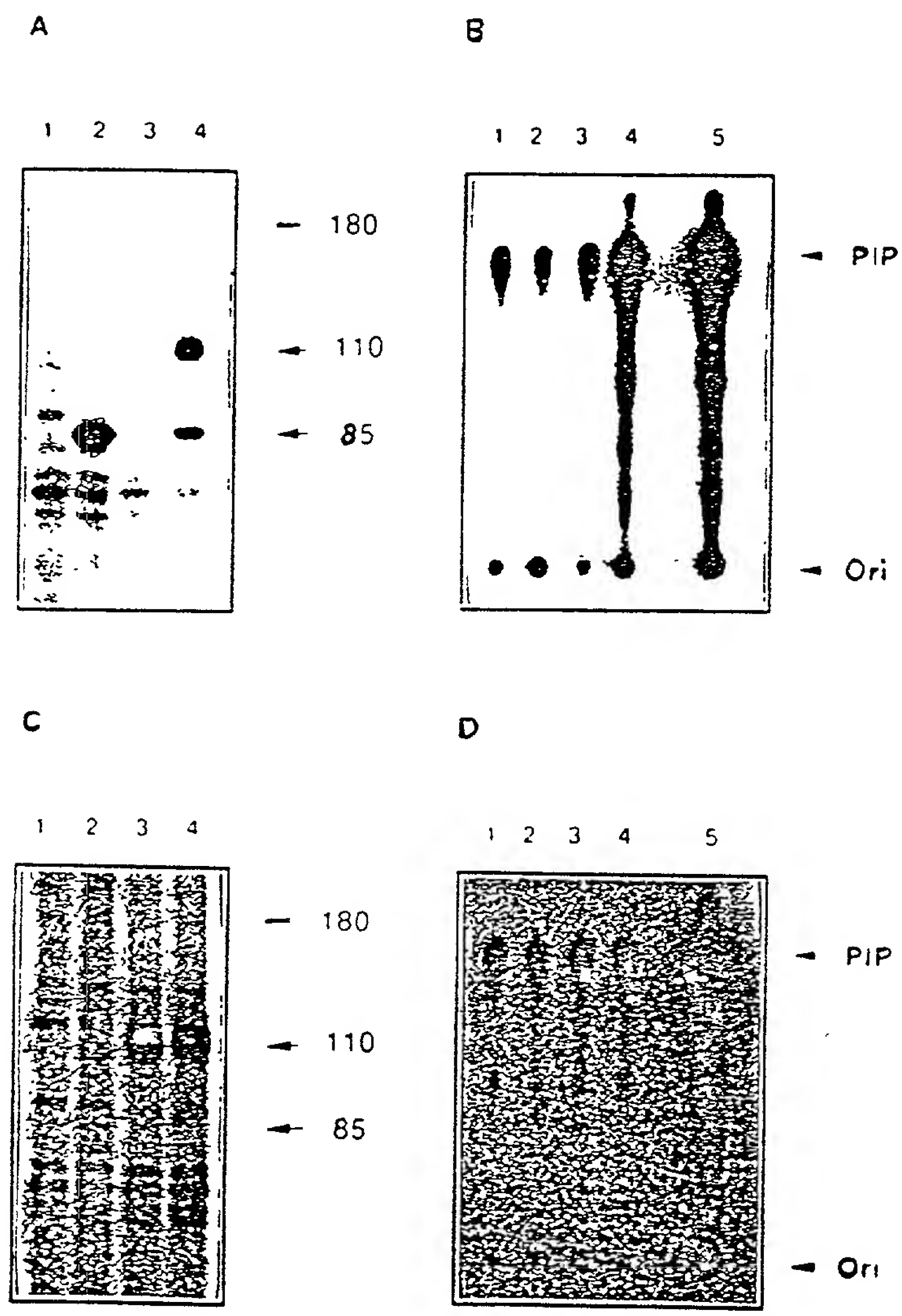


Figure 14

Figure 15


A
1 2 3 4
180
110
85
B
1 2 3 4 5
PIP
Ori
C
1 2 3 4
180
110
85
D
1 2 3 4 5
PIP
Ori

The human p110 cDNA nucleotide and deduced amino acid sequence.

```
    ATGCCTCCAAGACCATCATCAGGTGAACTGTGGGGCATCCACTTGATG
1   ---------+---------+---------+---------+--------    48
    TACGGAGGTTCTGGTAGTAGTCCACTTGACACCCCGTAGGTGAACTAC
    M  P  P  R  P  S  S  G  E  L  W  G  I  H  L  M

    CCCCCAAGAATCCTAGTGGAATGTTTACTACCAAATGGAATGATAGTG
49  -+---------+---------+---------+---------+------    96
    GGGGGTTCTTAGGATCACCTTACAAATGATGGTTTACCTTACTATCAC
    P  P  R  I  L  V  E  C  L  L  P  N  G  M  I  V

    ACTTTAGAATGCCTCCGTGAGGCTACATTAGTAACTATAAAGCATGAA
97  ---+---------+---------+---------+---------+----    144
    TGAAATCTTACGGAGGCACTCCGATGTAATCATTGATATTTCGTACTT
    T  L  E  C  L  R  E  A  T  L  V  T  I  K  H  E

    CTATTTAAAGAAGCAAGAAAATACCCTCTCCATCAACTTCTTCAAGAT
145 -----+---------+---------+---------+---------+--    192
    GATAAATTTCTTCGTTCTTTTATGGGAGAGGTAGTTGAAGAAGTTCTA
    L  F  K  E  A  R  K  Y  P  L  H  Q  L  L  Q  D
```

Figure 16

```
    GAATCTTCTTACATTTTCGTAAGTGTTACCCAAGAAGCAGAAAGGGAA
193 -------+---------+---------+---------+---------+ 240
    CTTAGAAGAATGTAAAAGCATTCACAATGGGTTCTTCGTCTTTCCCTT
    E  S  S  Y  I  F  V  S  V  T  Q  E  A  E  R  E

    GAATTTTTTGATGAAACAAGACGACTTTGTGATCTTCGGCTTTTTCAA
241 ---------+---------+---------+---------+-------- 288
    CTTAAAAAACTACTTTGTTCTGCTGAAACACTAGAAGCCGAAAAAGTT
    E  F  F  D  E  T  R  R  L  C  D  L  R  L  F  Q

    CCATTTTTAAAAGTAATTGAACCAGTAGGCAACCGTGAAGAAAAGATC
289 -+---------+---------+---------+---------+------ 336
    GGTAAAAATTTTCATTAACTTGGTCATCCGTTGGCACTTCTTTTCTAG
    P  F  L  K  V  I  E  P  V  G  N  R  E  E  K  I

    CTCAATCGAGAAATTGGTTTTGCTATCGGCATGCCAGTGTGCGAATTT
337 ---+---------+---------+---------+---------+---- 384
    GAGTTAGCTCTTTAACCAAAACGATAGCCGTACGGTCACACGCTTAAA
    L  N  R  E  I  G  F  A  I  G  M  P  V  C  E  F
```

FIG 16 (contd)

```
     GATATGGTTAAAGATCCTGAAGTACAGGACTTCCGAAGAAATATTCTT
385  -----+---------+---------+---------+---------+--  432
     CTATACCAATTTCTAGGACTTCATGTCCTGAAGGCTTCTTTATAAGAA
     D  M  V  K  D  P  E  V  Q  D  F  R  R  N  I  L

     AATGTTTGTAAAGAAGCTGTGGATCTTAGGGATCTTAATTCACCTCAT
433  -------+---------+---------+---------+---------+  480
     TTACAAACATTTCTTCGACACCTAGAATCCCTAGAATTAAGTGGAGTA
     N  V  C  K  E  A  V  D  L  R  D  L  N  S  P  H

     AGTAGAGCAATGTATGTCTATCCGCCACATGTAGAATCTTCACCAGAG
481  ---------+---------+---------+---------+--------  528
     TCATCTCGTTACATACAGATAGGCGGTGTACATCTTAGAAGTGGTCTC
     S  R  A  M  Y  V  Y  P  P  H  V  E  S  S  P  E

     CTGCCAAAGCACATATATAATAAATTGGATAGAGGCCAAATAATAGTG
529  -+---------+---------+---------+---------+------  576
     GACGGTTTCGTGTATATATTATTTAACCTATCTCCGGTTTATTATCAC
     L  P  K  H  I  Y  N  K  L  D  R  G  Q  I  I  V
```

FIG 16 (contd)

```
    GTGATTTGGGTAATAGTTTCTCCAAATAATGACAAGCAGAAGTATACT
577 ---+---------+---------+---------+---------+---- 624
    CACTAAACCCATTATCAAAGAGGTTTATTACTGTTCGTCTTCATATGA
    V  I  W  V  I  V  S  P  N  N  D  K  Q  K  Y  T

    CTGAAAATCAACCATGACTGTGTGCCAGAACAAGTAATTGCTGAAGCA
625 -----+---------+---------+---------+---------+-- 672
    GACTTTTAGTTGGTACTGACACACGGTCTTGTTCATTAACGACTTCGT
    L  K  I  N  H  D  C  V  P  E  Q  V  I  A  E  A

    ATCAGGAAAAAAACTAGAAGTATGTTGCTATCATCTGAACAATTAAAA
673 -------+---------+---------+---------+---------+ 720
    TAGTCCTTTTTTTGATCTTCATACAACGATAGTAGACTTGTTAATTTT
    I  R  K  K  T  R  S  M  L  L  S  S  E  Q  L  K

    CTCTGTGTTTTAGAATATCAGGGCAAGTACATTTTAAAAGTGTGTGGA
721 ---------+---------+---------+---------+-------- 768
    GAGACACAAAATCTTATAGTCCCGTTCATGTAAAATTTTCACACACCT
    L  C  V  L  E  Y  Q  G  K  Y  I  L  K  V  C  G
```

FIG 16 (contd)

```
     TGTGATGAATACTTCCTAGAAAAATATCCTCTGAGTCAGTATAAGTAT
769  -+---------+---------+---------+---------+------- 816
     ACACTACTTATGAAGGATCTTTTTATAGGAGACTCAGTCATATTCATA
     C  D  E  Y  F  L  E  K  Y  P  L  S  Q  Y  K  Y

     ATAAGAAGCTGTATAATGCTTGGGAGGATGCCCAATTTGAAGATGATG
817  ---+---------+---------+---------+---------+----- 864
     TATTCTTCGACATATTACGAACCCTCCTACGGGTTAAACTTCTACTAC
     I  R  S  C  I  M  L  G  R  M  P  N  L  K  M  M

     GCTAAAGAAAGCCTTTATTCTCAACTGCCAATGGACTGTTTTACAATG
865  -----+---------+---------+---------+---------+--- 912
     CGATTTCTTTCGGAAATAAGAGTTGACGGTTACCTGACAAAATGTTAC
     A  K  E  S  L  Y  S  Q  L  P  M  D  C  F  T  M

     CCATCTTATTCCAGACGCATTTCCACAGCTACACCATATATGAATGGA
913  -------+---------+---------+---------+---------+ 960
     GGTAGAATAAGGTCTGCGTAAAGGTGTCGATGTGGTATATACTTACCT
     P  S  Y  S  R  R  I  S  T  A  T  P  Y  M  N  G
```

FIG 16 (contd)

```
       GAAACATCTACAAAATCCCTTTGGGTTATAAATAGAGCACTCAGAATA
961    ---------+---------+---------+---------+--------   1008
       CTTTGTAGATGTTTTAGGGAAACCCAATATTTATCTCGTGAGTCTTAT
       E  T  S  T  K  S  L  W  V  I  N  R  A  L  R  I

       AAAATTCTTTGTGCAACCTACGTGAATCTAAATATTCGAGACATTGAC
1009   -+---------+---------+---------+---------+------   1056
       TTTTAAGAAACACGTTGGATGCACTTAGATTTATAAGCTCTGTAACTG
       K  I  L  C  A  T  Y  V  N  L  N  I  R  D  I  D

       AAGATTTATGTTCGAACAGGTATCTACCATGGAGGAGAACCCTTATGT
1057   ---+---------+---------+---------+---------+----   1104
       TTCTAAATACAAGCTTGTCCATAGATGGTACCTCCTCTTGGGAATACA
       K  I  Y  V  R  T  G  I  Y  H  G  G  E  P  L  C

       GACAATGTGAACACTCAAAGAGTACCTTGTTCCAATCCCAGGTGGAAT
1105   -----+---------+---------+---------+---------+--   1152
       CTGTTACACTTGTGAGTTTCTCATGGAACAAGGTTAGGGTCCACCTTA
       D  N  V  N  T  Q  R  V  P  C  S  N  P  R  W  N
```

FIG 16 (contd)

```
      GAATGGCTGAATTATGATATATACATTCCTGATCTTCCTCGTGCTGCT
1153  -------+---------+---------+---------+---------+  1200
      CTTACCGACTTAATACTATATATGTAAGGACTAGAAGGAGCACGACGA
      E  W  L  N  Y  D  I  Y  I  P  D  L  P  R  A  A

      CGACTTTGCCTTTCCATTTGCTCTGTTAAAGGCCGAAAGGGTGCTAAA
1201  ---------+---------+---------+---------+--------  1248
      GCTGAAACGGAAAGGTAAACGAGACAATTTCCGGCTTTCCCACGATTT
      R  L  C  L  S  I  C  S  V  K  G  R  K  G  A  K

      GAGGAACACTGTCCATTGGCATGGGGAAATATAAACTTGTTTGATTAC
1249  -+---------+---------+---------+---------+------  1296
      CTCCTTGTGACAGGTAACCGTACCCCTTTATATTTGAACAAACTAATG
      E  E  H  C  P  L  A  W  G  N  I  N  L  F  D  Y

      ACAGACACTCTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTA
1297  ---+---------+---------+---------+---------+----  1344
      TGTCTGTGAGATCATAGACCTTTTTACCGAAACTTAGAAACCGGTCAT
      T  D  T  L  V  S  G  K  M  A  L  N  L  W  P  V
```

FIG 16 (contd)

```
     CCTCATGGATTAGAAGATTTGCTGAACCCTATTGGTGTTACTGGATCA
1345 -----+---------+---------+---------+---------+-- 1392
     GGAGTACCTAATCTTCTAAACGACTTGGGATAACCACAATGACCTAGT
     P  H  G  L  E  D  L  L  N  P  I  G  V  T  G  S

     AATCCAAATAAAGAAACTCCATGCTTAGAGTTGGAGTTTGACTGGTTC
1393 -------+---------+---------+---------+---------+ 1440
     TTAGGTTTATTTCTTTGAGGTACGAATCTCAACCTCAAACTGACCAAG
     N  P  N  K  E  T  P  C  L  E  L  E  F  D  W  F

     AGCAGTGTGGTAAAGTTCCCAGATATGTCAGTGATTGAAGAGCATGCC
1441 ---------+---------+---------+---------+-------- 1488
     TCGTCACACCATTTCAAGGGTCTATACAGTCACTAACTTCTCGTACGG
     S  S  V  V  K  F  P  D  M  S  V  I  E  E  H  A

     AATTGGTCTGTATCCCGAGAAGCAGGATTTAGCTATTCCCACGCAGGA
1489 -+---------+---------+---------+---------+------ 1536
     TTAACCAGACATAGGGCTCTTCGTCCTAAATCGATAAGGGTGCGTCCT
     N  W  S  V  S  R  E  A  G  F  S  Y  S  H  A  G
```

FIG 16 (contd)

```
     CTGAGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAA
1537 ---+---------+---------+---------+---------+---- 1584
     GACTCATTGTCTGATCGATCTCTGTTACTTAATTCCCTTTTACTGTTT
     L  S  N  R  L  A  R  D  N  E  L  R  E  N  D  K

     GAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACT
1585 -----+---------+---------+---------+---------+-- 1632
     CTTGTCGAGTTTCGTTAAAGATGTGCTCTAGGAGAGAGACTTTAGTGA
     E  Q  L  K  A  I  S  T  R  D  P  L  S  E  I  T

     GAGCAGGAGAAAGATTTTCTATGGAGTCACAGACACTATTGTGTAACT
1633 -------+---------+---------+---------+---------+ 1680
     CTCGTCCTCTTTCTAAAAGATACCTCAGTGTCTGTGATAACACATTGA
     E  Q  E  K  D  F  L  W  S  H  R  H  Y  C  V  T

     ATCCCCGAAATTCTACCCAAATTGCTTCTGTCTGTTAAATGGAATTCT
1681 ---------+---------+---------+---------+-------- 1728
     TAGGGGCTTTAAGATGGGTTTAACGAAGACAGACAATTTACCTTAAGA
     I  P  E  I  L  P  K  L  L  L  S  V  K  W  N  S
```

FIG 16 (contd)

```
     AGAGATGAAGTAGCCCAGATGTATTGCTTGGTAAAAGATTGGCCTCCA
1729 -+---------+---------+---------+---------+------ 1776
     TCTCTACTTCATCGGGTCTACATAACGAACCATTTTCTAACCGGAGGT
     R  D  E  V  A  Q  M  Y  C  L  V  K  D  W  P  P

     ATCAAACCTGAACAGGCTATGGAACTTCTGGACTGTAATTACCCAGAT
1777 ---+---------+---------+---------+---------+---- 1824
     TAGTTTGGACTTGTCCGATACCTTGAAGACCTGACATTAATGGGTCTA
     I  K  P  E  Q  A  M  E  L  L  D  C  N  Y  P  D

     CCTATGGTTCGAGGTTTTGCTGTTCGGTGCTTGGAAAAATATTTAACA
1825 -----+---------+---------+---------+---------+-- 1872
     GGATACCAAGCTCCAAAACGACAAGCCACGAACCTTTTTATAAATTGT
     P  M  V  R  G  F  A  V  R  C  L  E  K  Y  L  T

     GATGACAAACTTTCTCAGTATTTAATTCAGCTAGTACAGGTCCTAAAA
1873 -------+---------+---------+---------+---------+ 1920
     CTACTGTTTGAAAGAGTCATAAATTAAGTCGATCATGTCCAGGATTTT
     D  D  K  L  S  Q  Y  L  I  Q  L  V  Q  V  L  K
```

FIG 16 (contd)

```
      TATGAACAATATTTGGATAACTTGCTTGTGAGATTTTTACTGAAGAAA
1921  ---------+---------+---------+---------+--------  1968
      ATACTTGTTATAAACCTATTGAACGAACACTCTAAAAATGACTTCTTT
      Y  E  Q  Y  L  D  N  L  L  V  R  F  L  L  K  K

      GCATTGACTAATCAAAGGATTGGGCACTTTTTCTTTTGGCATTTAAAA
1969  -+---------+---------+---------+---------+------  2016
      CGTAACTGATTAGTTTCCTAACCCGTGAAAAAGAAAACCGTAAATTTT
      A  L  T  N  Q  R  I  G  H  F  F  F  W  H  L  K

      TCTGAGATGCACAATAAAACAGTTAGCCAGAGGTTTGGCCTGCTTTTG
2017  ---+---------+---------+---------+---------+----  2064
      AGACTCTACGTGTTATTTTGTCAATCGGTCTCCAAACCGGACGAAAAC
      S  E  M  H  N  K  T  V  S  Q  R  F  G  L  L  L

      GAGTCCTATTGTCGTGCATGTGGGATGTATTTGAAGCACCTGAATAGG
2065  -----+---------+---------+---------+---------+--  2112
      CTCAGGATAACAGCACGTACACCCTACATAAACTTCGTGGACTTATCC
      E  S  Y  C  R  A  C  G  M  Y  L  K  H  L  N  R
```

FIG 16 (contd)

```
     CAAGTCGAGGCAATGGAAAAGCTCATTAACTTAACTGACATTCTCAAA
2113 -------+---------+---------+---------+---------+ 2160
     GTTCAGCTCCGTTACCTTTTCGAGTAATTGAATTGACTGTAAGAGTTT
     Q  V  E  A  M  E  K  L  I  N  L  T  D  I  L  K

     CAGGAGAGGAAGGATGAAACACAAAAGGTACAGATGAAGTTTTTAGTT
2161 ---------+---------+---------+---------+-------- 2208
     GTCCTCTCCTTCCTACTTTGTGTTTTCCATGTCTACTTCAAAAATCAA
     Q  E  R  K  D  E  T  Q  K  V  Q  M  K  F  L  V

     GAGCAAATGAGGCGACCAGATTTCATGGATGCCCTACAGGGCTTGCTG
2209 -+---------+---------+---------+---------+------ 2256
     CTCGTTTACTCCGCTGGTCTAAAGTACCTACGGGATGTCCCGAACGAC
     E  Q  M  R  R  P  D  F  M  D  A  L  Q  G  L  L

     TCTCCTCTAAACCCTGCTCATCAACTAGGAAACCTCAGGCTTAAAGAG
2257 ---+---------+---------+---------+---------+---- 2304
     AGAGGAGATTTGGGACGAGTAGTTGATCCTTTGGAGTCCGAATTTCTC
     S  P  L  N  P  A  H  Q  L  G  N  L  R  L  K  E
```

FIG 16 (contd)

```
     TGTCGAATTATGTCTTCTGCAAAAAGGCCACTGTGGTTGAATTGGGAG
2305 -----+---------+---------+---------+---------+-- 2352
     ACAGCTTAATACAGAAGACGTTTTTCCGGTGACACCAACTTAACCCTC
     C  R  I  M  S  S  A  K  R  P  L  W  L  N  W  E

     AACCCAGACATCATGTCAGAGTTACTGTTTCAGAACAATGAGATCATC
2353 -------+---------+---------+---------+---------+ 2400
     TTGGGTCTGTAGTACAGTCTCAATGACAAAGTCTTGTTACTCTAGTAG
     N  P  D  I  M  S  E  L  L  F  Q  N  N  E  I  I

     TTTAAAAATGGGGATGATTTACGGCAAGATATGCTAACACTTCAAATT
2401 ---------+---------+---------+---------+-------- 2448
     AAATTTTTACCCCTACTAAATGCCGTTCTATACGATTGTGAAGTTTAA
     F  K  N  G  D  D  L  R  Q  D  M  L  T  L  Q  I

     ATTCGTATTATGGAAAATATCTGGCAAAATCAAGGTCTTGATCTTCGA
2449 -+---------+---------+---------+---------+------ 2496
     TAAGCATAATACCTTTTATAGACCGTTTTAGTTCCAGAACTAGAAGCT
     I  R  I  M  E  N  I  W  Q  N  Q  G  L  D  L  R
```

FIG 16 (contd)

```
      ATGTTACCTTATGGTTGTCTGTCAATCGGTGACTGTGTGGGACTTATT
2497  ---+---------+---------+---------+---------+----  2544
      TACAATGGAATACCAACAGACAGTTAGCCACTGACACACCCTGAATAA
      M  L  P  Y  G  C  L  S  I  G  D  C  V  G  L  I

      GAGGTGGTGCGAAATTCTCACACTATTATGCAAATTCAGTGCAAAGGC
2545  -----+---------+---------+---------+---------+--  2592
      CTCCACCACGCTTTAAGAGTGTGATAATACGTTTAAGTCACGTTTCCG
      E  V  V  R  N  S  H  T  I  M  Q  I  Q  C  K  G

      GGCTTGAAAGGTGCACTGCAGTTCAACAGCCACACACTACATCAGTGG
2593  -------+---------+---------+---------+---------+  2640
      CCGAACTTTCCACGTGACGTCAAGTTGTCGGTGTGTGATGTAGTCACC
      G  L  K  G  A  L  Q  F  N  S  H  T  L  H  Q  W

      CTCAAAGACAAGAACAAAGGAGAAATATATGATGCAGCCATTGACCTG
2641  ---------+---------+---------+---------+--------  2688
      GAGTTTCTGTTCTTGTTTCCTCTTTATATACTACGTCGGTAACTGGAC
      L  K  D  K  N  K  G  E  I  Y  D  A  A  I  D  L
```

FIG 16 (contd)

```
     TTTACACGTTCATGTGCTGGATACTGTGTAGCTACCTTCATTTTGGGA
2689 -+---------+---------+---------+---------+------ 2736
     AAATGTGCAAGTACACGACCTATGACACATCGATGGAAGTAAAACCCT
     F  T  R  S  C  A  G  Y  C  V  A  T  F  I  L  G

     ATTGGAGATCGTCACAATAGTAACATCATGGTGAAAGACGATGGACAA
2737 ---+---------+---------+---------+---------+---- 2784
     TAACCTCTAGCAGTGTTATCATTGTAGTACCACTTTCTGCTACCTGTT
     I  G  D  R  H  N  S  N  I  M  V  K  D  D  G  Q

     CTGTTTCATATAGATTTTGGACACTTTTTGGATCACAAGAAGAAAAAA
2785 -----+---------+---------+---------+---------+-- 2832
     GACAAAGTATATCTAAAACCTGTGAAAAACCTAGTGTTCTTCTTTTTT
     L  F  H  I  D  F  G  H  F  L  D  H  K  K  K  K

     TTTGGTTATAAACGAGAACGTGTGCCATTTGTTTTGACACAGGATTTC
2833 -------+---------+---------+---------+---------+ 2880
     AAACCAATATTTGCTCTTGCACACGGTAAACAAAACTGTGTCCTAAAG
     F  G  Y  K  R  E  R  V  P  F  V  L  T  Q  D  F
```

FIG 16 (contd)

```
      TTAATAGTGATTAGTAAAGGAGCCCAAGAATGCACAAAGACAAGAGAA
2881  ---------+---------+---------+---------+--------  2928
      AATTATCACTAATCATTTCCTCGGGTTCTTACGTGTTTCTGTTCTCTT
      L  I  V  I  S  K  G  A  Q  E  C  T  K  T  R  E

      TTTGAGAGGTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTATTCGA
2929  -+---------+---------+---------+---------+------  2976
      AAACTCTCCAAAGTCCTCTACACAATGTTCCGAATAGATCGATAAGCT
      F  E  R  F  Q  E  M  C  Y  K  A  Y  L  A  I  R

      CAGCATGCCAATCTCTTCATAAATCTTTTCTCAATGATGCTTGGCTCT
2977  ---+---------+---------+---------+---------+----  3024
      GTCGTACGGTTAGAGAAGTATTTAGAAAAGAGTTACTACGAACCGAGA
      Q  H  A  N  L  F  I  N  L  F  S  M  M  L  G  S

      GGAATGCCAGAACTACAATCTTTTGATGACATTGCATACATTCGAAAG
3025  -----+---------+---------+---------+---------+--  3072
      CCTTACGGTCTTGATGTTAGAAAACTACTGTAACGTATGTAAGCTTTC
      G  M  P  E  L  Q  S  F  D  D  I  A  Y  I  R  K
```

FIG 16 (contd)

```
      ACCCTAGCCTTAGATAAAACTGAGCAAGAGGCTTTGGAGTATTTCATG
3073  --------+---------+---------+---------+---------+  3120
      TGGGATCGGAATCTATTTTGACTCGTTCTCCGAAACCTCATAAAGTAC
      T  L  A  L  D  K  T  E  Q  E  A  L  E  Y  F  M

      AAACAAATGAATGATGCACATCATGGTGGCTGGACAACAAAAATGGAT
3121  ---------+---------+---------+---------+--------  3168
      TTTGTTTACTTACTACGTGTAGTACCACCGACCTGTTGTTTTTACCTA
      K  Q  M  N  D  A  H  H  G  G  W  T  T  K  M  D

      TGGATCTTCCACACAATTAAACAGCATGCATTGAACTGAAAGATAACT
3169  -+---------+---------+---------+---------+------  3216
      ACCTAGAAGGTGTGTTAATTTGTCGTACGTAACTTGACTTTCTATTGA
      W  I  F  H  T  I  K  Q  H  A  L  N  *

      GAGAAAATGAAAGCTCACTCTGGATTCCACACTGCACTGTTAATAACT
3217  ---+---------+---------+---------+---------+----  3264
      CTCTTTTACTTTCGAGTGAGACCTAAGGTGTGACGTGACAATTATTGA
```

FIG 16 (contd)

```
     CTCAGCAGGCAAAGACCGATTGCATAGGAATTGCACAATCCATGAACA
3265 -----+---------+---------+---------+---------+--  3312
     GAGTCGTCCGTTTCTGGCTAACGTATCCTTAACGTGTTAGGTACTTGT

     GCATTAGATTTACAGCAAGAACAGAAATAAAATACTATATAATTTAAA
3313 -------+---------+---------+---------+---------+  3360
     CGTAATCTAAATGTCGTTCTTGTCTTTATTTTATGATATATTAAATTT

     TAATGTAAACGCAAACAGGGTTTGATAGCACTTAAACTAGTTCATTTC
3361 ---------+---------+---------+---------+--------  3408
     ATTACATTTGCGTTTGTCCCAAACTATCGTGAATTTGATCAAGTAAAG

     AAAA
3409 -+--  3412
     TTTT
```

FIG 16 (contd)

Alignment of human and bovine p110 cDNA nucleotide sequences.

Nucleotide Similarity: 96%

```
                    .         .         .         .         .
hum110   1 ATGCCTCCAAGACCATCATCAGGTGAACTGTGGGCATCCACTTGATGCC  50
           ||||||||||||||||||||||||||||||||||||||||||||||||||
bov110   1 ATGCCTCCAAGACCATCATCAGGTGAACTGTGGGCATCCACTTGATGCC  50

                    .         .         .         .         .
        51 CCCAAGAATCCTAGTGGAATGTTTACTACCAAATGGAATGATAGTGACTT 100
           ||||||||||||||| |||||||||||||||||||| |||||||||||||
        51 CCCAAGAATCCTAGTAGAATGTTTACTACCAAATGGGATGATAGTGACTT 100

                    .         .         .         .         .
       101 TAGAATGCCTCCGTGAGGCTACATTAGTAACTATAAAGCATGAACTATTT 150
           |||||||||||||||||||||| ||| |||| ||||||||||||||||||
       101 TAGAATGCCTCCGTGAGGCTACGTTAATAACGATAAAGCATGAACTATTT 150

                    .         .         .         .         .
       151 AAAGAAGCAAGAAAATACCCTCTCCATCAACTTCTTCAAGATGAATCTTC 200
           ||||||||||||||||||||||||||||||||||||||||||||||||||
       151 AAAGAAGCAAGAAAATACCCTCTCCATCAACTTCTTCAAGATGAATCTTC 200
```

Figure 17

```
                 .         .         .         .         .
201 TTACATTTTCGTAAGTGTTACCCAAGAAGCAGAAAGGAAGAATTTTTTG 250
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 TTACATTTTCGTAAGTGTTACCCAAGAAGCAGAAAGGAAGAATTTTTTG 250
                 .         .         .         .         .
251 ATGAAACAAGACGACTTTGTGATCTTCGGCTTTTTCAACCATTTTTAAAA 300
    ||||||||||||||||||||||| |||||||||||||||| |||||||||
251 ATGAAACAAGACGACTTTGTGACCTTCGGCTTTTTCAACCCTTTTTAAAA 300
                 .         .         .         .         .
301 GTAATTGAACCAGTAGGCAACCGTGAAGAAAAGATCCTCAATCGAGAAAT 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 GTAATTGAACCAGTAGGCAACCGTGAAGAAAAGATCCTCAATCGAGAAAT 350
                 .         .         .         .         .
351 TGGTTTTGCTATCGGCATGCCAGTGTGCGAATTTGATATGGTTAAAGATC 400
    ||||||||||||||||||||||||||| ||||| ||||||||||||||||
351 TGGTTTTGCTATCGGCATGCCAGTGTGTGAATTCGATATGGTTAAAGATC 400
                 .         .         .         .         .
401 CTGAAGTACAGGACTTCCGAAGAAATATTCTTAATGTTTGTAAAGAAGCT 450
    | ||||||||||||||||||||||||||||| ||||||||||||||||||
401 CAGAAGTACAGGACTTCCGAAGAAATATTCTCAATGTTTGTAAAGAAGCT 450
```

FIG 17 (contd)

```
451 GTGGATCTTAGGGATCTTAATTCACCTCATAGTAGAGCAATGTATGTCTA 500
    ||||||||||||||||||||||||||||||||||||||||||||||| ||
451 GTGGATCTTAGGGATCTTAATTCACCTCATAGTAGAGCAATGTATGTTTA 500

501 TCCGCCACATGTAGAATCTTCACCAGAGCTGCCAAAGCACATATATAATA 550
    ||| ||| ||||||||||||||||||| ||||||||||||||||||||||
501 TCCTCCAAATGTAGAATCTTCACCAGAACTGCCAAAGCACATATATAATA 550

551 AATTGGATAGAGGCCAAATAATAGTGGTGATTTGGTAATAGTTTCTCCA 600
    ||||||||| ||| ||||||||||||||||||||||||||||||||||||
551 AATTGGATAAAGGGCAAATAATAGTGGTGATTTGGTAATAGTTTCTCCA 600

601 AATAATGACAAGCAGAAGTATACTCTGAAAATCAACCATGACTGTGTGCC 650
    ||||||||||| ||||||||||||||||||||||||||||||||||||||
601 AATAATGACAAACAGAAGTATACTCTGAAAATCAACCATGACTGTGTGCC 650

651 AGAACAAGTAATTGCTGAAGCAATCAGGAAAAAAACTAGAAGTATGTTGC 700
    |||||||||||||||||||||||||||||||||||||| |||||||||||
651 AGAACAAGTAATTGCTGAAGCAATCAGGAAAAAAACTCGAAGTATGTTGC 700
```

FIG 17 (contd)

```
701 TATCATCTGAACAATTAAAACTCTGTGTTTTAGAATATCAGGCAAGTAC 750
    |||||||||||||| |||||||||||||||||||||||||||||||||| 
701 TATCATCTGAACAACTAAAACTCTGTGTTTTAGAATATCAGGCAAGTAT 750

751 ATTTTAAAAGTGTGTGGATGTGATGAATACTTCCTAGAAAAATATCCTCT 800
    ||||||||||||||||||||||||||||||||||||||||||||||||||
751 ATTTTAAAAGTGTGTGGATGTGATGAATACTTCCTAGAAAAATATCCTCT 800

801 GAGTCAGTATAAGTATATAAGAAGCTGTATAATGCTTGGAGGATGCCCA 850
    ||||||||||||||||||||||||||||||||||||||||||||||||||
801 GAGTCAGTATAAGTATATAAGAAGCTGTATAATGCTTGGAGGATGCCCA 850

851 ATTTGAAGATGATGGCTAAAGAAAGCCTTTATTCTCAACTGCCAATGGAC 900
    |||||| | ||||||||||||||||||| |||||||||||||||||||||
851 ATTTGATGCTGATGGCTAAAGAAAGCCTCTATTCTCAACTGCCAATGGAC 900

901 TGTTTTACAATGCCATCTTATTCCAGACGCATTTCCACAGCTACACCATA 950
    ||||||||||||||||| |||||||||||||| ||||||||||| |||||
901 TGTTTTACAATGCCATCATATTCCAGACGCATCTCCACAGCTACGCCATA 950
```

FIG 17 (contd)

```
                 .         .         .         .         .
 951 TATGAATGGAGAAACATCTACAAAATCCCTTTGGGTTATAAATAGAGCAC 1000
     ||||||||||||||||||||||||||||||||||||||||||||| ||||
 951 TATGAATGGAGAAACATCTACAAAATCCCTTTGGGTTATAAATAGTGCAC 1000
                 .         .         .         .         .
1001 TCAGAATAAAAATTCTTTGTGCAACCTACGTGAATCTAAATATTCGAGAC 1050
     |||||||||||||||||||||||||||| |||||| ||||||||||||||
1001 TCAGAATAAAAATTCTTTGTGCAACCTATGTGAATGTAAATATTCGAGAC 1050
                 .         .         .         .         .
1051 ATTGACAAGATTTATGTTCGAACAGGTATCTACCATGGAGGAGAACCCTT 1100
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1051 ATTGACAAGATTTATGTTCGAACAGGTATCTACCATGGAGGAGAACCCTT 1100
                 .         .         .         .         .
1101 ATGTGACAATGTGAACACTCAAAGAGTACCTTGTTCCAATCCCAGGTGGA 1150
     |||||| |||||||||||||||||||||||||||||||||||||||||||
1101 ATGTGATAATGTGAACACTCAAAGAGTACCTTGTTCCAATCCCAGGTGGA 1150
                 .         .         .         .         .
1151 ATGAATGGCTGAATTATGATATATACATTCCTGATCTTCCTCGTGCTGCT 1200
     |||||||||||||||| |||||||||||||||||||||||||||||||||
1151 ATGAATGGCTGAATTACGATATATACATTCCTGATCTTCCTCGTGCTGCT 1200
```

FIG 17 (contd)

```
              .         .         .         .         .
1201 CGACTTTGCCTTTCCATTTGCTCTGTTAAAGGCCGAAAGGGTGCTAAAGA 1250
     |||||||||||||||||||| |||||||||||||||||||||||||||||
1201 CGACTTTGCCTTTCCATTTGTTCTGTTAAAGGCCGAAAGGGTGCTAAAGA 1250
              .         .         .         .         .
1251 GGAACACTGTCCATTGGCATGGGAAATATAAACTTGTTTGATTACACAG 1300
     |||||||||||||||||| |||||||||||||||||||||||||||||||
1251 GGAACACTGTCCATTGGCCTGGGAAATATAAACTTGTTTGATTACACAG 1300
              .         .         .         .         .
1301 ACACTCTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTACCTCAT 1350
     | ||||||||||||||||||||||||||||||||||||||||||||||||
1301 ATACTCTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTACCTCAT 1350
              .         .         .         .         .
1351 GGATTAGAAGATTTGCTGAACCCTATTGGTGTTACTGGATCAAATCCAAA 1400
     ||| ||||||||||||||||||||||||||||||||||||||||||||||
1351 GGACTAGAAGATTTGCTGAACCCTATTGGTGTTACTGGATCAAATCCAAA 1400
              .         .         .         .         .
1401 TAAAGAAACTCCATGCTTAGAGTTGGAGTTTGACTGGTTCAGCAGTGTGG 1450
     ||||||||||||||| ||||||||||||||||||||||||||||||||||
1401 TAAAGAAACTCCATGTTTAGAGTTGGAGTTTGACTGGTTCAGCAGTGTGG 1450
```

FIG 17 (contd)

```
                   .         .         .         .         .
1451  TAAAGTTCCCAGATATGTCAGTGATTGAAGAGCATGCCAATTGGTCTGTA  1500
      ||||||| ||||||||||||||||||||||||||||||||||||||||||
1451  TAAAGTTTCCAGATATGTCAGTGATTGAAGAGCATGCCAATTGGTCTGTA  1500
                   .         .         .         .         .
1501  TCCCGAGAAGCAGGATTTAGCTATTCCCACGCAGGACTGAGTAACAGACT  1550
      ||||| |||||||||||||| |||||||| ||||||||||||||||||||
1501  TCCCGTGAAGCAGGATTTAGTTATTCCCATGCAGGACTGAGTAACAGACT  1550
                   .         .         .         .         .
1551  AGCTAGAGACAATGAATTAAGGGAAAATGACAAAGAACAGCTCAAAGCAA  1600
      ||||||||||||||||||||| |||||||| ||||||||||||  |||||
1551  AGCTAGAGACAATGAATTAAGAGAAAATGATAAAGAACAGCTCCGAGCAA  1600
                   .         .         .         .         .
1601  TTTCTACACGAGATCCTCTCTCTGAAATCACTGAGCAGGAGAAAGATTTT  1650
      ||| ||||||||||||||| ||||||||||||||||| ||||||||||||
1601  TTTGTACACGAGATCCTCTATCTGAAATCACTGAGCAAGAGAAAGATTTT  1650
                   .         .         .         .         .
1651  CTATGGAGTCACAGACACTATTGTGTAACTATCCCCGAAATTCTACCCAA  1700
      || ||||| |||||||||||||||||||||||||||||||||||||||||
1651  CTGTGGAGCCACAGACACTATTGTGTAACTATCCCCGAAATTCTACCCAA  1700
```

FIG 17 (contd)

```
                .         .         .         .         .
1701 ATTGCTTCTGTCTGTTAAATGGAATTCTAGAGATGAAGTAGCCCAGATGT 1750
     |||||||||||||||||||||||| ||||||||||||||||| |||||||
1701 ATTGCTTCTGTCTGTTAAATGGAACTCTAGAGATGAAGTAGCTCAGATGT 1750

                .         .         .         .         .
1751 ATTGCTTGGTAAAAGATTGGCCTCCAATCAAACCTGAACAGGCTATGGAA 1800
     | ||||||||||||||||||||||||||||| ||||||||||||||||| 
1751 ACTGCTTGGTAAAAGATTGGCCTCCAATCAAGCCTGAACAGGCTATGGAG 1800

                .         .         .         .         .
1801 CTTCTGGACTGTAATTACCCAGATCCTATGGTTCGAGGTTTTGCTGTTCG 1850
     ||||||||||| ||||||||||||||||||||||||||||||||||||||
1801 CTTCTGGACTGCAATTACCCAGATCCTATGGTTCGAGGTTTTGCTGTTCG 1850

                .         .         .         .         .
1851 GTGCTTGGAAAAATATTTAACAGATGACAAACTTTCTCAGTATTTAATTC 1900
     |||||| |||||||||||||||||||||||||||||||||||  ||||||
1851 GTGCTTAGAAAAATATTTAACAGATGACAAACTTTCTCAGTACCTAATTC 1900

                .         .         .         .         .
1901 AGCTAGTACAGGTCCTAAAATATGAACAATATTTGGATAACTTGCTTGTG 1950
     ||||||||||||| |||||||||||||| |||||||||||| ||||||||
1901 AGCTAGTACAGGTACTAAAATATGAACAGTATTTGGATAACCTGCTTGTG 1950
```

FIG 17 (contd)

```
                 .         .         .         .         .
1951 AGATTTTTACTGAAGAAAGCATTGACTAATCAAAGGATTGGGCACTTTTT 2000
     ||||||||||| || ||||| || |||||||||||||| || ||||||||
1951 AGATTTTTACTCAAAAAAGCGTTAACTAATCAAAGGATCGGTCACTTTTT 2000

                 .         .         .         .         .
2001 CTTTTGGCATTTAAAATCTGAGATGCACAATAAAACAGTTAGCCAGAGGT 2050
     |||||||||||||||||||||||||||||||||||||||||| |||||||
2001 CTTTTGGCATTTAAAATCTGAGATGCACAATAAAACAGTTAGTCAGAGGT 2050

                 .         .         .         .         .
2051 TTGGCCTGCTTTTTGGAGTCCTATTGTCGTGCATGTGGATGTATTTGAAG 2100
     |||||||||||||||||||||||||| ||||||||||||||||| |||||
2051 TTGGCCTGCTTTTTGGAGTCCTATTGCCGTGCATGTGGATGTATCTGAAG 2100

                 .         .         .         .         .
2101 CACCTGAATAGGCAAGTCGAGGCAATGGAAAAGCTCATTAACTTAACTGA 2150
     ||||| ||||||||||| ||||| |||||||||||||||||||| |||||
2101 CACCTTAATAGGCAAGTTGAGGCTATGGAAAAGCTCATTAACTTGACTGA 2150

                 .         .         .         .         .
2151 CATTCTCAAACAGGAGAGGAAGGATGAAACACAAAAGGTACAGATGAAGT 2200
     |||||||||||| |||| ||||||||||||||||||||||||||||||||
2151 CATTCTCAAACAAGAGAAGAAGGATGAAACACAAAAGGTACAGATGAAGT 2200
```

FIG 17 (contd)

```
              .         .         .         .         .
2201 TTTTAGTTGAGCAAATGAGGCGACCAGATTTCATGGATGCCCTACAGGGC 2250
     ||||||||||||||||| |||||||||||||||||||||| || ||||||
2201 TTTTAGTTGAGCAAATGCGGCGACCAGATTTCATGGATGCTCTCCAGGGC 2250
              .         .         .         .         .
2251 TTGCTGTCTCCTCTAAACCCTGCTCATCAACTAGGAAACCTCAGGCTTAA 2300
     || |||||||||||||||||||||||||| || ||||| ||||||||| |
2251 TTTCTGTCTCCTCTAAACCCTGCTCATCAGCTGGGAAATCTCAGGCTTGA 2300
              .         .         .         .         .
2301 AGAGTGTCGAATTATGTCTTCTGCAAAAAGGCCACTGTGGTTGAATTGGG 2350
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2301 AGAGTGTCGAATTATGTCTTCTGCAAAAAGGCCACTGTGGTTGAATTGGG 2350
              .         .         .         .         .
2351 AGAACCCAGACATCATGTCAGAGTTACTGTTTCAGAACAATGAGATCATC 2400
     |||||||||||||||||||||| ||||| |||||||||||||||||||||
2351 AGAACCCAGACATCATGTCAGAATTACTCTTTCAGAACAATGAGATCATC 2400
              .         .         .         .         .
2401 TTTAAAAAATGGGATGATTTACGGCAAGATATGCTAACACTTCAAATTAT 2450
     |||||||||||||||||||||||||||||||||||||| ||||| |||||
2401 TTTAAAAAATGGGATGATTTACGGCAAGATATGCTAACCCTTCAGATTAT 2450
```

FIG 17 (contd)

```
                  .         .         .         .         .
2451 TCGTATTATGGAAAATATCTGGCAAAATCAAGGTCTTGATCTTCGAATGT 2500
     ||| ||||||||||||||||||||||||||||||||||||||||||||||
2451 TCGCATTATGGAAAATATCTGGCAAAATCAAGGTCTTGATCTTCGAATGT 2500

                  .         .         .         .         .
2501 TACCTTATGGTTGTCTGTCAATCGGTGACTGTGTGGGACTTATTGAGGTG 2550
     |||||||||| |||||||||||||||||||||||||||||||| ||||||
2501 TACCTTATGGATGTCTGTCAATCGGTGACTGTGTGGGACTTATCGAGGTG 2550

                  .         .         .         .         .
2551 GTGCGAAATTCTCACACTATTATGCAAATTCAGTGCAAAGGCGGCTTGAA 2600
     ||| |||||||||||||||| ||||| |||||||| ||||| ||| ||||
2551 GTGAGAAATTCTCACACTATAATGCAGATTCAGTGTAAAGGAGGCCTGAA 2600

                  .         .         .         .         .
2601 AGGTGCACTGCAGTTCAACAGCCACACACTACATCAGTGGCTCAAAGACA 2650
     ||||||||||||||| |||||||||||||| |||||||||||||||||||
2601 AGGTGCACTGCAGTTTAACAGCCACACACTCCATCAGTGGCTCAAAGACA 2650

                  .         .         .         .         .
2651 AGAACAAAGGAGAAATATATGATGCAGCCATTGACCTGTTTACACGTTCA 2700
     ||||||| || |||||||||||||| ||||| ||  |||||||||| |||
2651 AGAACAAGGGGGAAATATATGATGCGGCCATCGATTTGTTTACACGATCA 2700
```

FIG 17 (contd)

```
                  .         .         .         .         .
2701 TGTGCTGGATACTGTGTAGCTACCTTCATTTTGGGAATTGGAGATCGTCA 2750
     ||||||||||| ||||| || |||||||||||||||||||||||||||||
2701 TGTGCTGGATATTGTGTTGCCACCTTCATTTTGGGAATTGGAGATCGTCA 2750

                  .         .         .         .         .
2751 CAATAGTAACATCATGGTGAAAGACGATGGACAACTGTTTCATATAGATT 2800
     ||||||||| |||||||| ||||| |||||||||||||||||||||||||
2751 CAATAGTAATATCATGGTTAAAGATGATGGACAACTGTTTCATATAGATT 2800

                  .         .         .         .         .
2801 TTGGACACTTTTTGGATCACAAGAAGAAAAAATTTGGTTATAAACGAGAA 2850
     ||||||||||||||||||||||||||||||||||||||||||||||||| 
2801 TTGGACACTTTTTGGATCACAAGAAGAAAAAATTTGGTTATAAACGAGAG 2850

                  .         .         .         .         .
2851 CGTGTGCCATTTGTTTTGACACAGGATTTCTTAATAGTGATTAGTAAAGG 2900
     || ||||| |||||||||||||| ||||||||||||||||||||||||||
2851 CGCGTGCCGTTTGTTTTGACACAAGATTTCTTAATAGTGATTAGTAAAGG 2900

                  .         .         .         .         .
2901 AGCCCAAGAATGCACAAAGACAAGAGAATTTGAGAGGTTTCAGGAGATGT 2950
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2901 AGCCCAAGAATGCACAAAGACAAGAGAATTTGAGAGGTTTCAGGAGATGT 2950
```

FIG 17 (contd)

```
2951 GTTACAAGGCTTATCTAGCTATTCGACAGCATGCCAATCTCTTCATAAAT 3000
     ||||||||||||||||||||||||| ||||||||||||||||||||||||
2951 GTTACAAGGCTTATCTAGCTATTCGGCAGCATGCCAATCTCTTCATAAAT 3000

3001 CTTTTCTCAATGATGCTTGGCTCTGGAATGCCAGAACTACAATCTTTTGA 3050
     |||||||||||||||||||||||||||||||||||||| |||||||||||
3001 CTTTTCTCAATGATGCTTGGCTCTGGAATGCCAGAACTGCAATCTTTTGA 3050

3051 TGACATTGCATACATTCGAAAGACCCTAGCCTTAGATAAAACTGAGCAAG 3100
     ||| |||||||||||||||||||||||||| |||||||||||||||||||
3051 TGATATTGCATACATTCGAAAGACCCTAGCTTTAGATAAAACTGAGCAAG 3100

3101 AGGCTTTGGAGTATTTCATGAAACAAATGAATGATGCACATCATGGTGGC 3150
     |||||||||||||||||||||||||||||||||||||||| |||||||||
3101 AGGCTTTGGAGTATTTCATGAAACAAATGAATGATGCACACCATGGTGGC 3150

3151 TGGACAACAAAAATGGATTGGATCTTCCACACAATTAAACAGCATGCATT 3200
     |||||||||||||||||||||||||||||||||||||| |||||||| ||
3151 TGGACAACAAAAATGGATTGGATCTTCCACACAATTAAGCAGCATGCTTT 3200
```

FIG 17 (contd)

```
         .         .         .         .
3201 GAACTGAAAGATAACTGAGAAAATGAAAGCTCACTCTGGA
     |||||||
3201 GAACTGA.................................
```

FIG 17 (contd)

The alignment between human and bovine p110 proteins.

```
          10        20        30        40        50        60
h MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLVTIKHELFKEARKYPLHQ
  ||||||||||||||||||||||||||||||||||||||||||:|||||||||||||||||
b MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLITIKHELFKEARKYPLHQ
          10        20        30        40        50        60

          70        80        90       100       110       120
h LLQDESSYIFVSVTQEAEREEFFDETRRLCDLRLFQPFLKVIEPVGNREEKILNREIGFA
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b LLQDESSYIFVSVTQEAEREEFFDETRRLCDLRLFQPFLKVIEPVGNREEKILNREIGFA
          70        80        90       100       110       120

         130       140       150       160       170       180
h IGMPVCEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPHVESSPELPKH
  |||||||||||||||||||||||||||||||||||||||||||||||||:||||||||||
b IGMPVCEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPNVESSPELPKH
         130       140       150       160       170       180
```

Figure 18

```
           190       200       210       220       230       240
h IYNKLDRGQIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSMLLSSEQLK
  ||||||:|||||||||||||||||||||||||||||||||||||||||||||||||||||
b IYNKLDKGQIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSMLLSSEQLK
           190       200       210       220       230       240

           250       260       270       280       290       300
h LCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNLKMMAKESLYSQLPMD
  |||||||||||||||||||||||||||||||||||||||||||||::|||||||||||||
b LCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNLMLMAKESLYSQLPMD
           250       260       270       280       290       300

           310       320       330       340       350       360
h CFTMPSYSRRISTATPYMNGETSTKSLWVINRALRIKILCATYVNLNIRDIDKIYVRTGI
  |||||||||||||||||||||||||||||||:|||||||||||||:||||||||||||||
b CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGI
           310       320       330       340       350       360
```

FIG 18 (contd)

```
          370       380       390       400       410       420
h YHGGEPLCDNVNTQRVPCSNPRWNEWLNYDIYIPDLPRAARLCLSICSVKGRKGAKEEHC
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b YHGGEPLCDNVNTQRVPCSNPRWNEWLNYDIYIPDLPRAARLCLSICSVKGRKGAKEEHC
          370       380       390       400       410       420

          430       440       450       460       470       480
h PLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGSNPNKETPCLELEFDWF
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b PLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGSNPNKETPCLELEFDWF
          430       440       450       460       470       480

          490       500       510       520       530       540
h SSVVKFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTRDPL
  |||||||||||||||||||||||||||||||||||||||||||||||||||:||:|||||
b SSVVKFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLRAICTRDPL
          490       500       510       520       530       540
```

FIG 18 (contd)

```
           550       560       570       580       590       600
h SEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQAME
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b SEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQAME
           550       560       570       580       590       600

           610       620       630       640       650       660
h LLDCNYPDPMVRGFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQYLDNLLVRFLLKKALTN
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b LLDCNYPDPMVRGFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQYLDNLLVRFLLKKALTN
           610       620       630       640       650       660

           670       680       690       700       710       720
h QRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILK
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b QRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILK
           670       680       690       700       710       720
```

FIG 18 (contd)

```
          730       740       750       760       770       780
h QERKDETQKVQMKFLVEQMRRPDFMDALQGLLSPLNPAHQLGNLRLKECRIMSSAKRPLW
  ||:|||||||||||||||||||||||||||:|||||||||||||||:|||||||||||||
b QEKKDETQKVQMKFLVEQMRRPDFMDALQGFLSPLNPAHQLGNLRLEECRIMSSAKRPLW
          730       740       750       760       770       780

          790       800       810       820       830       840
h LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLS
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLS
          790       800       810       820       830       840

          850       860       870       880       890       900
h IGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLFTRS
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b IGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLFTRS
          850       860       870       880       890       900
```

FIG 18 (contd)

```
          910       920       930       940       950       960
h CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDF
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDF
          910       920       930       940       950       960

          970       980       990      1000      1010      1020
h LIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIA
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b LIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIA
          970       980       990      1000      1010      1020

         1030      1040      1050      1060      1070      1080
h YIRKTLALDKTEQEALEYFMKQMNDAHHGGWTTKMDWIFHTIKQHALNXKITEKMKAHSG
  ||||||||||||||||||||||||||||||||||||||||||||||||
b YIRKTLALDKTEQEALEYFMKQMNDAHHGGWTTKMDWIFHTIKQHALNX
         1030      1040      1050      1060
```

FIG 18 (contd)

Figure 19. The predicted amino acid sequence of human p110 cDNA.

1	MPPRPSSGEL	WGIHLMPPRI	LVECLLPNGM	IVTLECLREA	TLVTIKHELF
51	KEARKYPLHQ	LLQDESSYIF	VSVTQEAERE	EFFDETRRLC	DLRLFQPFLK
101	VIEPVGNREE	KILNREIGFA	IGMPVCEFDM	VKDPEVQDFR	RNILNVCKEA
151	VDLRDLNSPH	SRAMYVYPPH	VESSPELPKH	IYNKLDRGQI	IVVIWVIVSP
201	NNDKQKYTLK	INHDCVPEQV	IAEAIRKKTR	SMLLSSEQLK	LCVLEYQGKY
251	ILKVCGCDEY	FLEKYPLSQY	KYIRSCIMLG	RMPNLKMMAK	ESLYSQLPMD
301	CFTMPSYSRR	ISTATPYMNG	ETSTKSLWVI	NRALRIKILC	ATYVNLNIRD
351	IDKIYVRTGI	YHGGEPLCDN	VNTQRVPCSN	PRWNEWLNYD	IYIPDLPRAA
401	RLCLSICSVK	GRKGAKEEHC	PLAWGNINLF	DYTDTLVSGK	MALNLWPVPH
451	GLEDLLNPIG	VTGSNPNKET	PCLELEFDWF	SSVVKFPDMS	VIEEHANWSV

501	SREAGFSYSH	AGLSNRLARD	NELRENDKEQ	LKAISTRDPL	SEITEQEKDF
551	LWSHRHYCVT	IPEILPKLLL	SVKWNSRDEV	AQMYCLVKDW	PPIKPEQAME
601	LLDCNYPDPM	VRGFAVRCLE	KYLTDDKLSQ	YLIQLVQVLK	YEQYLDNLLV
651	RFLLKKALTN	QRIGHFFFWH	LKSEMHNKTV	SQRFGLLLES	YCRACGMYLK
701	HLNRQVEAME	KLINLTDILK	QERKDETQKV	QMKFLVEQMR	RPDFMDALQG
751	LLSPLNPAHQ	LGNLRLKECR	IMSSAKRPLW	LNWENPDIMS	ELLFQNNEII
801	FKNGDDLRQD	MLTLQIIRIM	ENIWQNQGLD	LRMLPYGCLS	IGDCVGLIEV
851	VRNSHTIMQI	QCKGGLKGAL	QFNSHTLHQW	LKDKNKGEIY	DAAIDLFTRS
901	CAGYCVATFI	LGIGDRHNSN	IMVKDDGQLF	HIDFGHFLDH	KKKKFGYKRE
951	RVPFVLTQDF	LIVISKGAQE	CTKTREFERF	QEMCYKAYLA	IRQHANLFIN
1001	LFSMMLGSGM	PELQSFDDIA	YIRKTLALDK	TEQEALEYFM	KQMNDAHHGG
1051	WTTKMDWIFH	TIKQHALN*			

FIG 19 (contd)

The human PITR-c partial cDNA nucleotide and deduced amino acid sequence.

```
1    GGAGACGACTTGCGACAGGATCAACTTATTCTTCAAATCATTTCACTC
     GlyAspAspLeuArgGlnAspGlnLeuIleLeuGlnIleIleSerLeu

49   ATGGACAAGCTGTTACGGAAAGAAAATCTGGACTTGAAATTGACACCT
     MetAspLysLeuLeuArgLysGluAsnLeuAspLeuLysLeuThrPro

97   TATAAGGTGTTAGCCACCAGTACAAAACATGGCTTCATGCAGtTTATC
     TyrLysValLeuAlaThrSerThrLysHisGlyPheMetGlnPheIle

145  CAGTCAGTtCCTGTGGCTGAaGTTCTTGATACAGAGGGAAGCATTCAG
     GlnSerValProValAlaGluValLeuAspThrGluGlySerIleGln

193  AACTTTTTTAGAAAATATGCACCAAGTGAGAATGGGCCAAATGGGATT
     AsnPhePheArgLysTyrAlaProSerGluAsnGlyProAsnGlyIle

241  AGTGCTGAGGTCATGGACACTtACGTTAAAAGCTGTGCTGGATATTGC
     SerAlaGluValMetAspThrTyrValLysSerCysAlaGlyTyrCys

289  GTGATCACCTATATACTTGGAGTTGGAGACAGGCACCTGGATAACCTT
     ValIleThrTyrIleLeuGlyValGlyAspArgHisLeuAspAsnLeu
```

Figure 20

337 TTGCTAACcAAAACAGGCAAACTCTTCCACATCGATTTCGGcCAC
LeuLeuThrLysThrGlyLysLeuPheHisIleAspPheGlyHis

FIG 20 (contd)

The human PITR-f partial cDNA nucleotide and deduced amino acid sequence.

```
  1  GGGGATGACTTACGGCAGGACATGCTAACGCTGCAGATGATTCGCATC
     GlyAspAspLeuArgGlnAspMetLeuThrLeuGlnMetIleArgIle

 49  ATGAGCAAGATCTGGGTCCAGGAGGGGCTGGACATGCGCATGGTCATC
     MetSerLysIleTrpValGlnGluGlyLeuAspMetArgMetValIle

 97  TTCCGCTGCTTCTCCACCGGCCGGGGCAGAGGGATGGTGGAGATGATC
     PheArgCysPheSerThrGlyArgGlyArgGlyMetValGluMetIle

145  CCTAATGCTGAGACCCTGCGTAAGATCCAGGTGGAGCATGGGGTGACc
     ProAsnAlaGluThrLeuArgLysIleGlnValGluHisGlyValThr

193  GGCTCGTTCAAGGACCGGCCCCTGGCAGACCGGCTGCAGAAACACAAC
     GlySerPheLysAspArgProLeuAlaAspArgLeuGlnLysHisAsn

241  CCTGGGGAGGACGAGTATGAGAAGGCTGTGGaGAACTTTATCTACTCC
     ProGlyGluAspGluTyrGluLysAlaValGluAsnPheIleTyrSer

289  TGCGCTGGCTGCTGCGTGGCCACGTACGTCTTGGGCATCTGTGACCga
     CysAlaGlyCysCysValAlaThrTyrValLeuGlyIleCysAspArg
```

Figure 21

```
337  CATAATGACAACATCATGCTGAAGACCACTGGTCACATGTTCCACATC
     HisAsnAspAsnIleMetLeuLysThrThrGlyHisMetPheHisIle

385  GACTTCGGC
     AspPheGly
```

FIG 21 (contd)

ALIGNMENT OF HUMAN P110, HUMAN PI-3 KINASE RELATED GENES PITR-C AND PITR-F AND YEAST PI-3 KINASE VPS34.

In upper case are shown amino acids that are conserved in 3 or more of the proteins. Underlined are the residues involved in ATP binding.

```
          1                                                50
vps34     GDDLRQDqLvvQIIslMnellknEnvDLkLtPYkiLaTGpqeGaIEfIpN
PITR-c    GDDLRQDqLiLQIIslMdkllrkEnLDLkLtPYkvLaTstkhGFmqfIqs
hump110   GDDLRQDmLtLQIIriMeniwqnqgLDLrMlPYgcLsiGdcvGLIEvVrN
PITR-f    GDDLRQDmLtLQmIriMskiwvqEgLDMrMviFrcFsTGrgrGMVEmIpN
Consensus GDDLRQD-L-LQII--M------E-LDL---PY--L-TG---G-IE-I-N

          51                                               100
vps34     dtlasilskyhGIlGy.......LklhypdeNatlgVqgwvlDnFVkSCA
PITR-c    vpvaevldtegsIqnf.......FrkYapseNgpngIsaevmDtYVkSCA
hump110   shtimqiqckgGlkGalqfnshtLhqWlkdkNkge.IydaaiDlFtrSCA
PITR-f    aetlrkiqvehGVtGs..fkdrpLadrlqkhNpgedeyekavEnFIySCA
Consensus -----------GI-G--------L-------N----I-----D-FV-SCA
```

Figure 22

```
               101                             133
vps34          GYCViTYILGVGDRHlDNlLvtpdGhFFHaDFG
PITR-c         GYCViTYILGVGDRHlDNlLltktGkLFHIDFG
hump110        GYCVaTFILGIGDRHnsNiMvkddGqLFHIDFG
PITR-f         GCCVaTYVLGIcDRHnDNiMlkttGhMFHIDFG
Consensus      GYCV-TYILG-GDRH-DN------G-LFHIDFG
```

FIG 22 (contd)

Figure 23 A

MAPPING THE P85 BINDING SITE ON P110

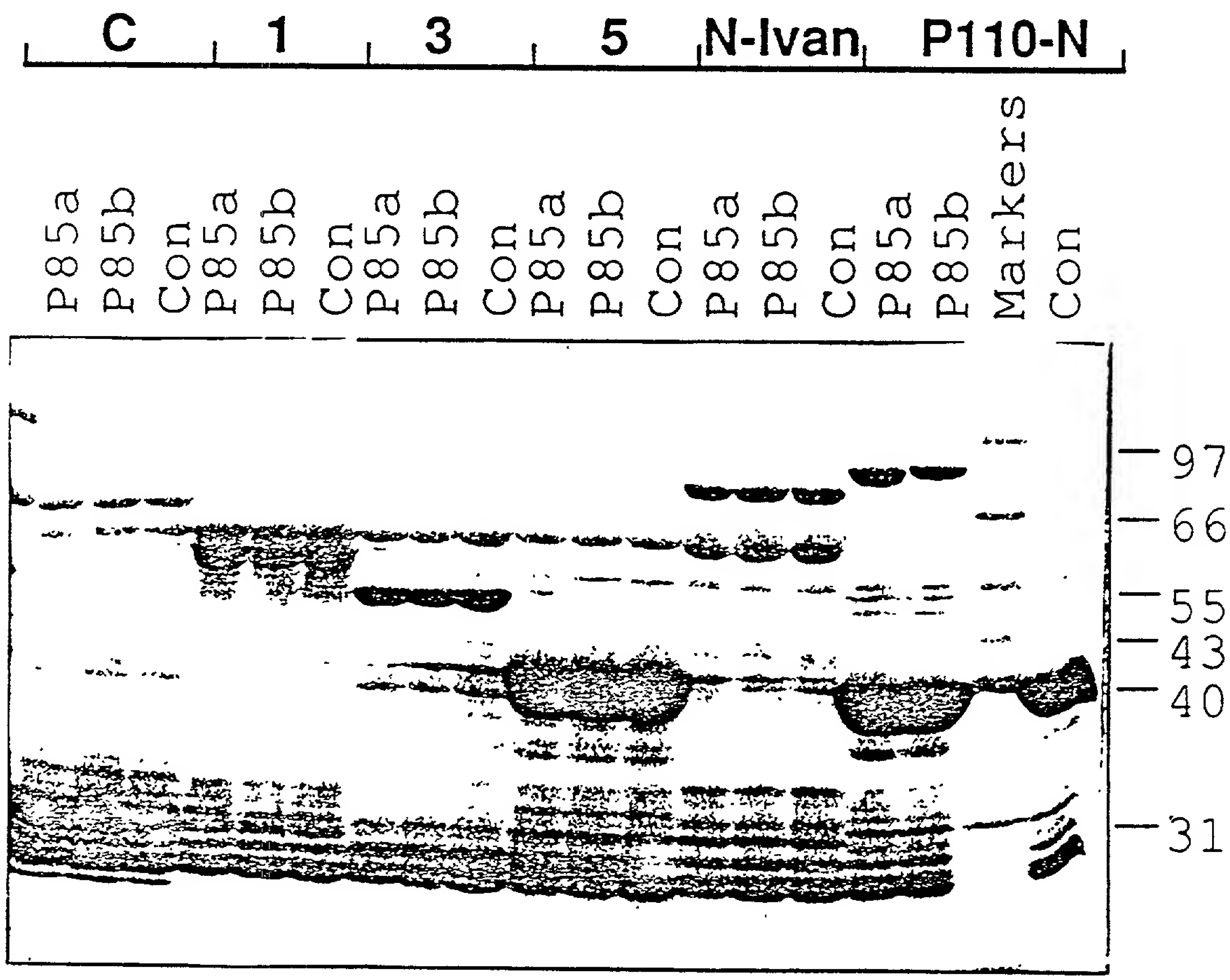


Figure 23 B

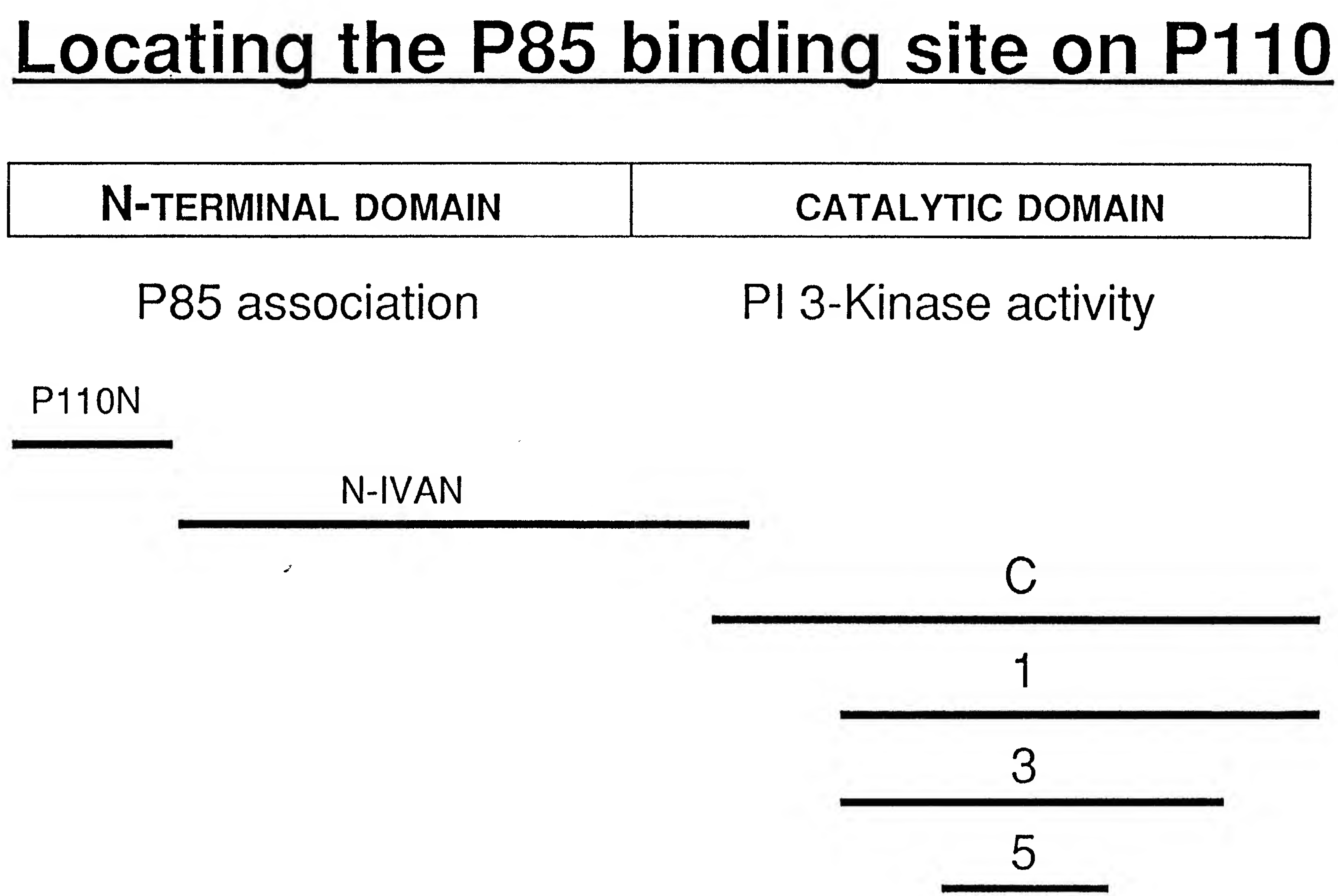

Locating the P85 binding site on P110
N-TERMINAL DOMAIN
CATALYTIC DOMAIN
P85 association
PI 3-Kinase activity
P110N
N-IVAN
C
1
3
5

Figure 24

Mapping of the P85 Binding Site on P110

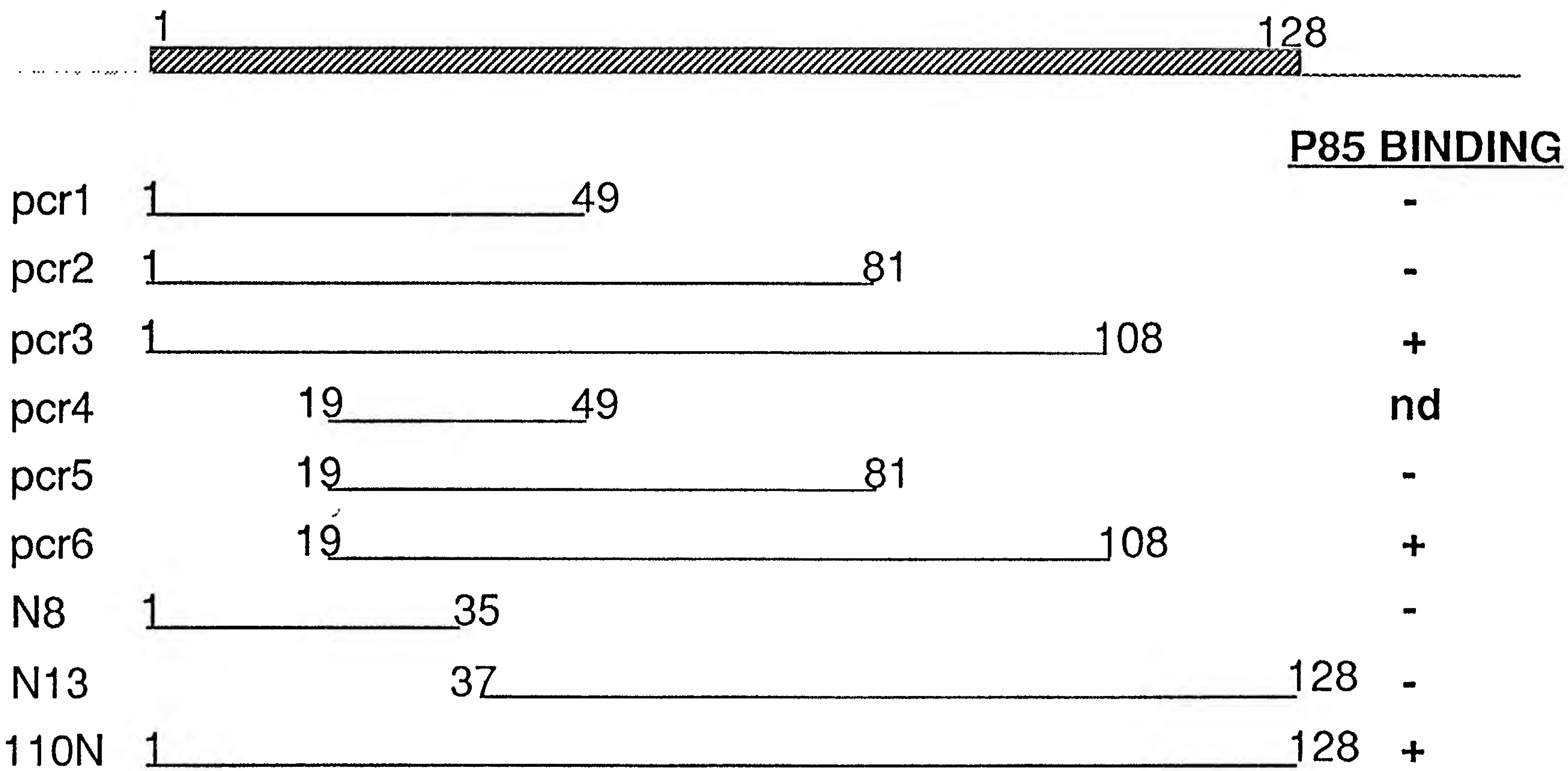


Figure 25 A

MAPPING THE P85 BINDING SITE ON P110

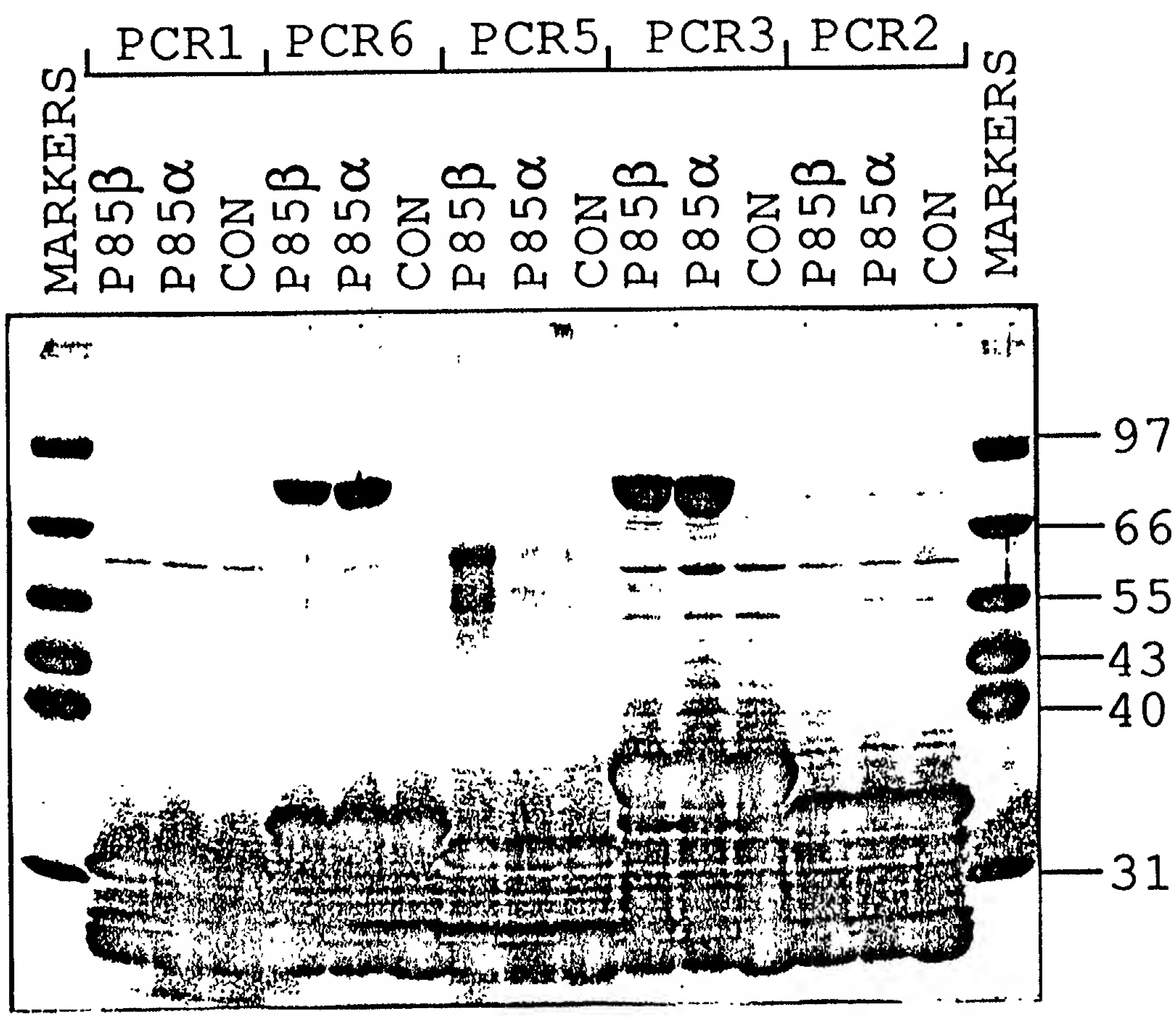
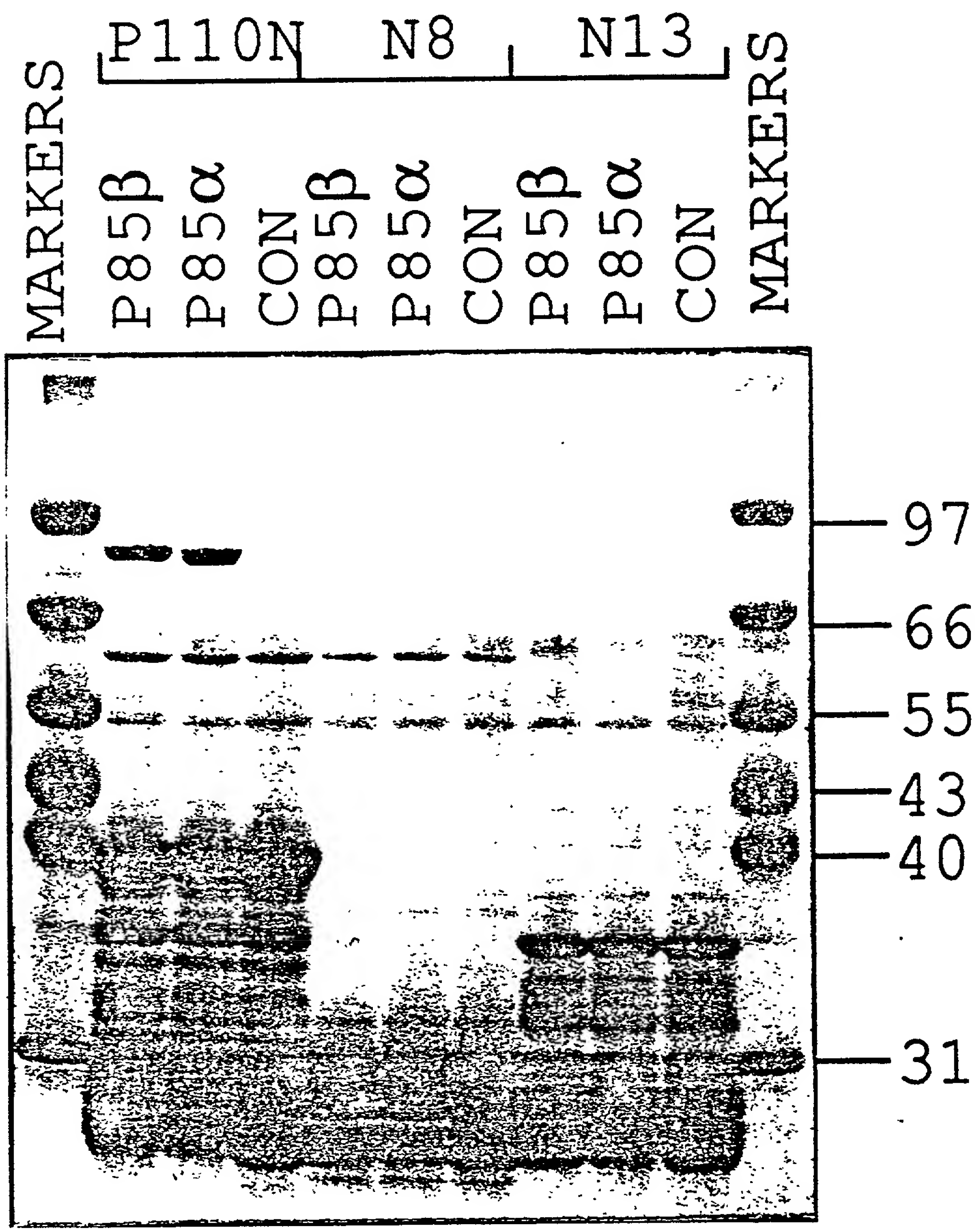


Figure 25 B

MAPPING THE P85 BINDING SITEON P110 (2)



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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under CFR 1.10 on the date indicated above, and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231
FULBRIGHT & JAWORSKI L.L.P.

By: Denise Squires

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Hiles, et al.
Serial No.	:	Divisional of Serial No. 09/085,957
Filed	:	May 27, 1998
For	:	Method for Determining Expression of a PI3 Kinase Gene
Group Art Unit	:	Not Yet Assigned
Examiner	:	Not Yet Assigned

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

June 3, 1999

LETTER RE: SEQUENCES

This application is a divisional of Serial No. 09/085,957, filed May 27, 1998. Applicants ask that the computer readable form of sequence information filed in that case on May 27, 1998, be transferred to this case, and that the accompanying paper copy of sequence information be used to replace the paper copy filed.

The undersigned hereby asserts that, to the best of his knowledge the accompanying CRF and paper copy of sequence information are identical to each other and to the sequence information in this application as filed. No new matter is presented.

Respectfully submitted,

FULBRIGHT & JAWORSKI L.L.P.

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579519.1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: POLYPEPTIDES HAVING KINASE ACTIVITY, THEIR PREPARATION AND USE

(iii) NUMBER OF SEQUENCES: 50

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(C) CITY: New York
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/162,081
(B) FILING DATE: February 7, 1994
(C) CLASSIFICATION: 435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/GB93/00761
(B) FILING DATE: 13 April 1993

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Gly Glu Ser Asp Gly Gly Tyr Met Asp Met Ser Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Asp Met Ser Lys Asp Glu Ser Val Asp Tyr Val Pro Met Leu Asp Met
1               5                   10                  15

Lys
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Cys Asp Glu Ser Val Asp Tyr Val Pro Met Leu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ala Arg Asp Ile Met Arg Asp Ser Asn Tyr Ile Ser Lys Gly Ser Thr
1 5 10 15

Phe

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Glu Phe Cys Pro Asp Pro Leu Tyr Glu Val Met Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Arg Arg Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Arg Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Thr Leu Ala Tyr Pro Val Tyr Ala Gln Gln Arg Arg
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Thr Leu Ala His Pro Val Arg Ala Pro Gly Pro Gly Pro Pro Ala Ala
1               5                   10                  15

Arg
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Tyr Xaa Xaa Met
1
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Asp Trp Ile Phe His Thr
1   5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AARATGGAYT GGATHTTYCA YAC 23

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Asp Asp Gly Gln Leu Phe His Ile Asp Phe Gly His Phe
1   5   10

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GATGATGGCC ARCTGTTYCA YATWGAYTTT GGCCA 35

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AATTCACACA CTGGCATGCC GAT 23

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GACTCGAGTC GACATCGATT TTTTTTTTTT TTTTT 35

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TTTAAGCTTA GGCATTCTAA AGTCACTATC ATCCC 35

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GACTCGAGTC GACATCGA 18

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CAGGCCTGGC TTCCTGT 17

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AACCAGGCTC AACTGTT 17

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TGCTGTAAAT TCTAATGCTG 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTATTTCATG AAACAAATGA 20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Cys Lys Met Asp Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AAGGATCAGA ACAATGCCT 19

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

AGGCTTTCTT TAGCCATCA 19

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Gly Asp Asp Leu Arg Gln Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GGNGAYGAYY TRCGNCARGA 20

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Phe His Ile Asp Phe Gly His Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

RAARTGCCRA ARTCDATRTG RAA 23

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Glu Glu Glu Glu Glu Tyr Met Pro Met Xaa Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Asp Asp Asp Asp Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3412 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single or double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..3204
(D) OTHER INFORMATION: /standard_name= "CDS"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ATG CCT CCA AGA CCA TCA TCA GGT GAA CTG TGG GGC ATC CAC TTG ATG 48
Met Pro Pro Arg Pro Ser Ser Gly Glu Leu Trp Gly Ile His Leu Met
1 5 10 15

CCC CCA AGA ATC CTA GTG GAA TGT TTA CTA CCA AAT GGA ATG ATA GTG 96
Pro Pro Arg Ile Leu Val Glu Cys Leu Leu Pro Asn Gly Met Ile Val
20 25 30

ACT TTA GAA TGC CTC CGT GAG GCT ACA TTA GTA ACT ATA AAG CAT GAA 144
Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Val Thr Ile Lys His Glu
35 40 45

CTA TTT AAA GAA GCA AGA AAA TAC CCT CTC CAT CAA CTT CTT CAA GAT 192
Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu Leu Gln Asp
50 55 60

GAA TCT TCT TAC ATT TTC GTA AGT GTT ACC CAA GAA GCA GAA AGG GAA 240
Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala Glu Arg Glu
65 70 75 80

GAA TTT TTT GAT GAA ACA AGA CGA CTT TGT GAT CTT CGG CTT TTT CAA 288
Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg Leu Phe Gln
85 90 95

```
CCA TTT TTA AAA GTA ATT GAA CCA GTA GGC AAC CGT GAA GAA AAG ATC      336
Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu Glu Lys Ile
            100                 105                 110

CTC AAT CGA GAA ATT GGT TTT GCT ATC GGC ATG CCA GTG TGC GAA TTT      384
Leu Asn Arg Glu Ile Gly Phe Ala Ile Gly Met Pro Val Cys Glu Phe
        115                 120                 125

GAT ATG GTT AAA GAT CCT GAA GTA CAG GAC TTC CGA AGA AAT ATT CTT      432
Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg Asn Ile Leu
    130                 135                 140

AAT GTT TGT AAA GAA GCT GTG GAT CTT AGG GAT CTT AAT TCA CCT CAT      480
Asn Val Cys Lys Glu Ala Val Asp Leu Arg Asp Leu Asn Ser Pro His
145                 150                 155                 160

AGT AGA GCA ATG TAT GTC TAT CCG CCA CAT GTA GAA TCT TCA CCA GAG      528
Ser Arg Ala Met Tyr Val Tyr Pro Pro His Val Glu Ser Ser Pro Glu
                165                 170                 175

CTG CCA AAG CAC ATA TAT AAT AAA TTG GAT AGA GGC CAA ATA ATA GTG      576
Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Arg Gly Gln Ile Ile Val
            180                 185                 190

GTG ATT TGG GTA ATA GTT TCT CCA AAT AAT GAC AAG CAG AAG TAT ACT      624
Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln Lys Tyr Thr
        195                 200                 205

CTG AAA ATC AAC CAT GAC TGT GTG CCA GAA CAA GTA ATT GCT GAA GCA      672
Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile Ala Glu Ala
    210                 215                 220

ATC AGG AAA AAA ACT AGA AGT ATG TTG CTA TCA TCT GAA CAA TTA AAA      720
Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu Gln Leu Lys
225                 230                 235                 240

CTC TGT GTT TTA GAA TAT CAG GGC AAG TAC ATT TTA AAA GTG TGT GGA      768
Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys Val Cys Gly
                245                 250                 255

TGT GAT GAA TAC TTC CTA GAA AAA TAT CCT CTG AGT CAG TAT AAG TAT      816
Cys Asp Glu Tyr Phe Leu Glu Lys Tyr Pro Leu Ser Gln Tyr Lys Tyr
            260                 265                 270
```

ATA AGA AGC TGT ATA ATG CTT GGG AGG ATG CCC AAT TTG AAG ATG ATG 864
Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu Lys Met Met
275 280 285

GCT AAA GAA AGC CTT TAT TCT CAA CTG CCA ATG GAC TGT TTT ACA ATG 912
Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys Phe Thr Met
290 295 300

CCA TCT TAT TCC AGA CGC ATT TCC ACA GCT ACA CCA TAT ATG AAT GGA 960
Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr Met Asn Gly
305 310 315 320

GAA ACA TCT ACA AAA TCC CTT TGG GTT ATA AAT AGA GCA CTC AGA ATA 1008
Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Arg Ala Leu Arg Ile
325 330 335

AAA ATT CTT TGT GCA ACC TAC GTG AAT CTA AAT ATT CGA GAC ATT GAC 1056
Lys Ile Leu Cys Ala Thr Tyr Val Asn Leu Asn Ile Arg Asp Ile Asp
340 345 350

AAG ATT TAT GTT CGA ACA GGT ATC TAC CAT GGA GGA GAA CCC TTA TGT 1104
Lys Ile Tyr Val Arg Thr Gly Ile Tyr His Gly Gly Glu Pro Leu Cys
355 360 365

GAC AAT GTG AAC ACT CAA AGA GTA CCT TGT TCC AAT CCC AGG TGG AAT 1152
Asp Asn Val Asn Thr Gln Arg Val Pro Cys Ser Asn Pro Arg Trp Asn
370 375 380

GAA TGG CTG AAT TAT GAT ATA TAC ATT CCT GAT CTT CCT CGT GCT GCT 1200
Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro Arg Ala Ala
385 390 395 400

CGA CTT TGC CTT TCC ATT TGC TCT GTT AAA GGC CGA AAG GGT GCT AAA 1248
Arg Leu Cys Leu Ser Ile Cys Ser Val Lys Gly Arg Lys Gly Ala Lys
405 410 415

GAG GAA CAC TGT CCA TTG GCA TGG GGA AAT ATA AAC TTG TTT GAT TAC 1296
Glu Glu His Cys Pro Leu Ala Trp Gly Asn Ile Asn Leu Phe Asp Tyr
420 425 430

ACA GAC ACT CTA GTA TCT GGA AAA ATG GCT TTG AAT CTT TGG CCA GTA 1344
Thr Asp Thr Leu Val Ser Gly Lys Met Ala Leu Asn Leu Trp Pro Val
435 440 445

CCT CAT GGA TTA GAA GAT TTG CTG AAC CCT ATT GGT GTT ACT GGA TCA 1392

Pro His Gly Leu Glu Asp Leu Leu Asn Pro Ile Gly Val Thr Gly Ser
450 455 460

AAT CCA AAT AAA GAA ACT CCA TGC TTA GAG TTG GAG TTT GAC TGG TTC 1440
Asn Pro Asn Lys Glu Thr Pro Cys Leu Glu Leu Glu Phe Asp Trp Phe
465 470 475 480

AGC AGT GTG GTA AAG TTC CCA GAT ATG TCA GTG ATT GAA GAG CAT GCC 1488
Ser Ser Val Val Lys Phe Pro Asp Met Ser Val Ile Glu Glu His Ala
485 490 495

AAT TGG TCT GTA TCC CGA GAA GCA GGA TTT AGC TAT TCC CAC GCA GGA 1536
Asn Trp Ser Val Ser Arg Glu Ala Gly Phe Ser Tyr Ser His Ala Gly
500 505 510

CTG AGT AAC AGA CTA GCT AGA GAC AAT GAA TTA AGG GAA AAT GAC AAA 1584
Leu Ser Asn Arg Leu Ala Arg Asp Asn Glu Leu Arg Glu Asn Asp Lys
515 520 525

GAA CAG CTC AAA GCA ATT TCT ACA CGA GAT CCT CTC TCT GAA ATC ACT 1632
Glu Gln Leu Lys Ala Ile Ser Thr Arg Asp Pro Leu Ser Glu Ile Thr
530 535 540

GAG CAG GAG AAA GAT TTT CTA TGG AGT CAC AGA CAC TAT TGT GTA ACT 1680
Glu Gln Glu Lys Asp Phe Leu Trp Ser His Arg His Tyr Cys Val Thr
545 550 555 560

ATC CCC GAA ATT CTA CCC AAA TTG CTT CTG TCT GTT AAA TGG AAT TCT 1728
Ile Pro Glu Ile Leu Pro Lys Leu Leu Leu Ser Val Lys Trp Asn Ser
565 570 575

AGA GAT GAA GTA GCC CAG ATG TAT TGC TTG GTA AAA GAT TGG CCT CCA 1776
Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp Trp Pro Pro
580 585 590

ATC AAA CCT GAA CAG GCT ATG GAA CTT CTG GAC TGT AAT TAC CCA GAT 1824
Ile Lys Pro Glu Gln Ala Met Glu Leu Leu Asp Cys Asn Tyr Pro Asp
595 600 605

CCT ATG GTT CGA GGT TTT GCT GTT CGG TGC TTG GAA AAA TAT TTA ACA 1872
Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys Tyr Leu Thr
610 615 620

GAT GAC AAA CTT TCT CAG TAT TTA ATT CAG CTA GTA CAG GTC CTA AAA 1920
Asp Asp Lys Leu Ser Gln Tyr Leu Ile Gln Leu Val Gln Val Leu Lys
625 630 635 640

TAT GAA CAA TAT TTG GAT AAC TTG CTT GTG AGA TTT TTA CTG AAG AAA 1968
Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu Leu Lys Lys
645 650 655

GCA TTG ACT AAT CAA AGG ATT GGG CAC TTT TTC TTT TGG CAT TTA AAA 2016
Ala Leu Thr Asn Gln Arg Ile Gly His Phe Phe Phe Trp His Leu Lys
660 665 670

TCT GAG ATG CAC AAT AAA ACA GTT AGC CAG AGG TTT GGC CTG CTT TTG 2064
Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly Leu Leu Leu
675 680 685

GAG TCC TAT TGT CGT GCA TGT GGG ATG TAT TTG AAG CAC CTG AAT AGG 2112
Glu Ser Tyr Cys Arg Ala Cys Gly Met Tyr Leu Lys His Leu Asn Arg
690 695 700

CAA GTC GAG GCA ATG GAA AAG CTC ATT AAC TTA ACT GAC ATT CTC AAA 2160
Gln Val Glu Ala Met Glu Lys Leu Ile Asn Leu Thr Asp Ile Leu Lys
705 710 715 720

CAG GAG AGG AAG GAT GAA ACA CAA AAG GTA CAG ATG AAG TTT TTA GTT 2208
Gln Glu Arg Lys Asp Glu Thr Gln Lys Val Gln Met Lys Phe Leu Val
725 730 735

GAG CAA ATG AGG CGA CCA GAT TTC ATG GAT GCC CTA CAG GGC TTG CTG 2256
Glu Gln Met Arg Arg Pro Asp Phe Met Asp Ala Leu Gln Gly Leu Leu
740 745 750

TCT CCT CTA AAC CCT GCT CAT CAA CTA GGA AAC CTC AGG CTT AAA GAG 2304
Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg Leu Lys Glu
755 760 765

TGT CGA ATT ATG TCT TCT GCA AAA AGG CCA CTG TGG TTG AAT TGG GAG 2352
Cys Arg Ile Met Ser Ser Ala Lys Arg Pro Leu Trp Leu Asn Trp Glu
770 775 780

AAC CCA GAC ATC ATG TCA GAG TTA CTG TTT CAG AAC AAT GAG ATC ATC 2400
Asn Pro Asp Ile Met Ser Glu Leu Leu Phe Gln Asn Asn Glu Ile Ile
785 790 795 800

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TTT AAA AAT GGG GAT GAT TTA CGG CAA GAT ATG CTA ACA CTT CAA ATT     2448
Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Ile
                805                 810                 815

ATT CGT ATT ATG GAA AAT ATC TGG CAA AAT CAA GGT CTT GAT CTT CGA     2496
Ile Arg Ile Met Glu Asn Ile Trp Gln Asn Gln Gly Leu Asp Leu Arg
            820                 825                 830

ATG TTA CCT TAT GGT TGT CTG TCA ATC GGT GAC TGT GTG GGA CTT ATT     2544
Met Leu Pro Tyr Gly Cys Leu Ser Ile Gly Asp Cys Val Gly Leu Ile
        835                 840                 845

GAG GTG GTG CGA AAT TCT CAC ACT ATT ATG CAA ATT CAG TGC AAA GGC     2592
Glu Val Val Arg Asn Ser His Thr Ile Met Gln Ile Gln Cys Lys Gly
    850                 855                 860

GGC TTG AAA GGT GCA CTG CAG TTC AAC AGC CAC ACA CTA CAT CAG TGG     2640
Gly Leu Lys Gly Ala Leu Gln Phe Asn Ser His Thr Leu His Gln Trp
865                 870                 875                 880

CTC AAA GAC AAG AAC AAA GGA GAA ATA TAT GAT GCA GCC ATT GAC CTG     2688
Leu Lys Asp Lys Asn Lys Gly Glu Ile Tyr Asp Ala Ala Ile Asp Leu
                885                 890                 895

TTT ACA CGT TCA TGT GCT GGA TAC TGT GTA GCT ACC TTC ATT TTG GGA     2736
Phe Thr Arg Ser Cys Ala Gly Tyr Cys Val Ala Thr Phe Ile Leu Gly
            900                 905                 910

ATT GGA GAT CGT CAC AAT AGT AAC ATC ATG GTG AAA GAC GAT GGA CAA     2784
Ile Gly Asp Arg His Asn Ser Asn Ile Met Val Lys Asp Asp Gly Gln
        915                 920                 925

CTG TTT CAT ATA GAT TTT GGA CAC TTT TTG GAT CAC AAG AAG AAA AAA     2832
Leu Phe His Ile Asp Phe Gly His Phe Leu Asp His Lys Lys Lys Lys
    930                 935                 940

TTT GGT TAT AAA CGA GAA CGT GTG CCA TTT GTT TTG ACA CAG GAT TTC     2880
Phe Gly Tyr Lys Arg Glu Arg Val Pro Phe Val Leu Thr Gln Asp Phe
945                 950                 955                 960

TTA ATA GTG ATT AGT AAA GGA GCC CAA GAA TGC ACA AAG ACA AGA GAA     2928
Leu Ile Val Ile Ser Lys Gly Ala Gln Glu Cys Thr Lys Thr Arg Glu
                965                 970                 975
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TTT GAG AGG TTT CAG GAG ATG TGT TAC AAG GCT TAT CTA GCT ATT CGA 2976
Phe Glu Arg Phe Gln Glu Met Cys Tyr Lys Ala Tyr Leu Ala Ile Arg
980 985 990

CAG CAT GCC AAT CTC TTC ATA AAT CTT TTC TCA ATG ATG CTT GGC TCT 3024
Gln His Ala Asn Leu Phe Ile Asn Leu Phe Ser Met Met Leu Gly Ser
995 1000 1005

GGA ATG CCA GAA CTA CAA TCT TTT GAT GAC ATT GCA TAC ATT CGA AAG 3072
Gly Met Pro Glu Leu Gln Ser Phe Asp Asp Ile Ala Tyr Ile Arg Lys
1010 1015 1020

ACC CTA GCC TTA GAT AAA ACT GAG CAA GAG GCT TTG GAG TAT TTC ATG 3120
Thr Leu Ala Leu Asp Lys Thr Glu Gln Glu Ala Leu Glu Tyr Phe Met
1025 1030 1035 1040

AAA CAA ATG AAT GAT GCA CAT CAT GGT GGC TGG ACA ACA AAA ATG GAT 3168
Lys Gln Met Asn Asp Ala His His Gly Gly Trp Thr Thr Lys Met Asp
1045 1050 1055

TGG ATC TTC CAC ACA ATT AAA CAG CAT GCA TTG AAC TGAAAGATAA 3214
Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn
1060 1065

CTGAGAAAAT GAAAGCTCAC TCTGGACACT ACACTGCACT GTTAATAACT CTCAGCAGGC 3274

AAAGACCGAT TGCATAGGAA TTGCACAATC CATGAACAGC ATTAGATTTA CAGCAAGAAC 3334

AGAAATAAAA TACTATATAA TTTAAATAAT GTAAACGCAA ACAGGGTTTG ATAGCACTTA 3394

AACTAGTTCA TTTCAAAA 3412

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 868 amino acids residues
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Asn Ile Thr Phe Cys Val Ser Gln Asp Leu Asp Val Pro Leu Lys Val
1 5 10 15

Lys Ile Lys Ser Leu Glu Gly His Lys Pro Leu Leu Lys Pro Ser Gln
20 25 30

Lys Ile Leu Asn Pro Glu Leu Met Leu Ile Gly Ser Asn Val Phe Pro
35 40 45

Ser Ser Asp Leu Ile Val Ser Leu Gln Val Phe Asp Lys Glu Arg Asn
50 55 60

Arg Asn Leu Thr Leu Pro Ile Tyr Thr Pro Tyr Ile Pro Phe Arg Asn
65 70 75 80

Ser Arg Thr Trp Asp Tyr Trp Leu Thr Leu Pro Ile Arg Ile Lys Gln
85 90 95

Leu Thr Phe Ser Ser His Leu Arg Ile Ile Leu Trp Glu Tyr Asn Gly
100 105 110

Ser Lys Gln Ile Pro Phe Phe Asn Leu Glu Thr Ser Ile Phe Asn Leu
115 120 125

Lys Asp Cys Thr Leu Lys Arg Gly Phe Glu Ser Leu Lys Phe Arg Tyr
130 135 140

Asp Val Ile Asp His Cys Glu Val Val Thr Asp Asn Lys Asp Gln Glu
145 150 155 160

Asn Leu Asn Lys Tyr Phe Gln Gly Glu Phe Thr Arg Leu Pro Trp Leu
165 170 175

Asp Glu Ile Thr Ile Ser Lys Leu Arg Lys Gln Arg Glu Asn Arg Thr
180 185 190

Trp Pro Gln Gly Thr Phe Val Leu Asn Leu Glu Phe Pro Met Leu Glu
195 200 205

Leu Pro Val Val Phe Ile Glu Arg Glu Ile Met Asn Thr Gln Met Asn
210 215 220

Ile Pro Thr Leu Lys Asn Asn Pro Gly Leu Ser Thr Asp Leu Arg Glu
225 230 235 240

Pro Asn Arg Asn Asp Pro Gln Ile Lys Ile Ser Leu Gly Asp Lys Tyr
245 250 255

His Ser Thr Leu Lys Phe Tyr Asp Pro Asp Gln Pro Asn Asn Asp Pro
260 265 270

Ile Glu Glu Lys Tyr Arg Arg Leu Glu Arg Ala Ser Lys Asn Ala Asn
275 280 285

Leu Asp Lys Gln Val Lys Pro Asp Ile Lys Lys Arg Asp Tyr Leu Asn
290 295 300

Lys Ile Ile Asn Tyr Pro Pro Gly Thr Lys Leu Thr Ala His Glu Lys
305 310 315 320

Gly Ser Ile Trp Lys Tyr Arg Tyr Tyr Leu Met Asn Asn Lys Lys Ala
325 330 335

Leu Thr Lys Leu Leu Gln Ser Thr Asn Leu Arg Glu Glu Ser Glu Arg
340 345 350

Val Glu Val Leu Glu Leu Met Asp Ser Trp Ala Glu Ile Asp Ile Asp
355 360 365

Asp Ala Leu Glu Leu Leu Gly Ser Thr Phe Lys Asn Leu Ser Val Arg
370 375 380

Ser Tyr Ala Val Asn Arg Leu Lys Lys Ala Ser Asp Lys Glu Leu Glu
385 390 395 400

Leu Tyr Leu Leu Gln Leu Val Glu Ala Val Cys Phe Glu Asn Leu Ser
405 410 415

Thr Phe Ser Asp Lys Ser Asn Ser Glu Phe Thr Ile Val Asp Ala Val
420 425 430

Ser Ser Gln Lys Leu Ser Gly Asp Ser Met Leu Leu Ser Thr Ser His
435 440 445

Ala Asn Gln Lys Leu Leu Lys Ser Ile Ser Ser Glu Ser Glu Thr Ser
450 455 460

Gly Thr Glu Ser Leu Pro Ile Val Ile Ser Pro Leu Ala Glu Phe Leu
465 470 475 480

Ile Arg Arg Ala Leu Val Asn Pro Arg Leu Gly Ser Phe Phe Tyr Trp
485 490 495

Tyr Leu Lys Ser Glu Ser Glu Asp Lys Pro Tyr Leu Asp Gln Ile Leu
500 505 510

Ser Ser Phe Trp Ser Arg Leu Asp Lys Lys Ser Arg Asn Ile Leu Asn
515 520 525

Asp Gln Val Arg Leu Ile Asn Val Leu Arg Glu Cys Cys Glu Thr Ile
530 535 540

Lys Arg Leu Lys Asp Thr Thr Ala Lys Lys Met Glu Leu Leu Val His
545 550 555 560

Leu Leu Glu Thr Lys Val Arg Pro Leu Val Lys Val Arg Pro Ile Ala
565 570 575

Leu Pro Leu Asp Pro Asp Val Leu Ile Cys Asp Val Cys Pro Glu Thr
580 585 590

Ser Lys Val Phe Lys Ser Ser Leu Ser Pro Leu Lys Ile Thr Phe Lys
595 600 605

Thr Thr Leu Asn Gln Pro Tyr His Leu Met Phe Lys Val Gly Asp Asp
610 615 620

Leu Arg Gln Asp Gln Leu Val Val Gln Ile Ile Ser Leu Met Asn Glu
625 630 635 640

Leu Leu Lys Asn Glu Asn Val Asp Leu Lys Leu Thr Pro Tyr Lys Ile
645 650 655

Leu Ala Thr Gly Pro Gln Glu Gly Ala Ile Glu Phe Ile Pro Asn Asp
660 665 670

Thr Leu Ala Ser Ile Leu Ser Lys Tyr His Gly Ile Leu Gly Tyr Leu
675 680 685

Lys Leu His Tyr Pro Asp Glu Asn Ala Thr Leu Gly Val Gln Gly Trp
690 695 700

Val Leu Asp Asn Phe Val Lys Ser Cys Ala Gly Tyr Cys Val Ile Thr
705 710 715 720

Tyr Ile Leu Gly Val Gly Asp Arg His Leu Asp Asn Leu Leu Val Thr
725 730 735

Pro Asp Gly His Phe Phe His Ala Asp Phe Gly Tyr Ile Leu Gly Gln
740 745 750

Asp Pro Lys Pro Phe Pro Pro Leu Met Lys Leu Pro Pro Gln Ile Ile
755 760 765

Glu Ala Phe Gly Gly Ala Glu Ser Ser Asn Tyr Asp Lys Phe Arg Ser
770 775 780

Tyr Cys Phe Val Ala Tyr Ser Ile Leu Arg Arg Asn Ala Gly Leu Ile
785 790 795 800

Leu Asn Leu Phe Glu Leu Met Lys Thr Ser Asn Ile Pro Asp Ile Arg
805 810 815

Ile Asp Pro Asn Gly Ala Ile Leu Arg Val Arg Glu Arg Phe Asn Leu
820 825 830

Asn Met Ser Glu Glu Asp Ala Thr Val His Phe Gln Asn Leu Ile Asn
835 840 845

Asp Ser Val Asn Ala Leu Leu Pro Ile Val Ile Asp His Leu His Asn
850 855 860

Leu Ala Gln Tyr
865

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3240 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

ATGCCTCCAA GACCATCATC AGGTGAACTG TGGGGCATCC ACTTGATGCC CCCAAGAATC 60

CTAGTGGAAT GTTTACTACC AAATGGAATG ATAGTGACTT TAGAATGCCT CCGTGAGGCT 120

ACATTAGTAA CTATAAAGCA TGAACTATTT AAAGAAGCAA GAAAATACCC TCTCCATCAA 180

CTTCTTCAAG ATGAATCTTC TTACATTTTC GTAAGTGTTA CCCAAGAAGC AGAAAGGGAA 240

GAATTTTTTG ATGAAACAAG ACGACTTTGT GATCTTCGGC TTTTTCAACC ATTTTTAAAA 300

GTAATTGAAC CAGTAGGCAA CCGTGAAGAA AAGATCCTCA ATCGAGAAAT TGGTTTTGCT 360

ATCGGCATGC CAGTGTGCGA ATTTGATATG GTTAAAGATC CTGAAGTACA GGACTTCCGA 420
AGAAATATTC TTAATGTTTG TAAAGAAGCT GTGGATCTTA GGGATCTTAA TTCACCTCAT 480
AGTAGAGCAA TGTATGTCTA TCCGCCACAT GTAGAATCTT CACCAGAGCT GCCAAAGCAC 540
ATATATAATA AATTGGATAG AGGCCAAATA ATAGTGGTGA TTTGGGTAAT AGTTTCTCCA 600
AATAATGACA AGCAGAAGTA TACTCTGAAA ATCAACCATG ACTGTGTGCC AGAACAAGTA 660
ATTGCTGAAG CAATCAGGAA AAAAACTAGA AGTATGTTGC TATCATCTGA ACAATTAAAA 720
CTCTGTGTTT TAGAATATCA GGGCAAGTAC ATTTTAAAAG TGTGTGGATG TGATGAATAC 780
TTCCTAGAAA AATATCCTCT GAGTCAGTAT AAGTATATAA GAAGCTGTAT AATGCTTGGG 840
AGGATGCCCA ATTTGAAGAT GATGGCTAAA GAAAGCCTTT ATTCTCAACT GCCAATGGAC 900
TGTTTTACAA TGCCATCTTA TTCCAGACGC ATTTCCACAG CTACACCATA TATGAATGGA 960
GAAACATCTA CAAAATCCCT TTGGGTTATA AATAGAGCAC TCAGAATAAA AATTCTTTGT 1020
GCAACCTATG TGAATGTAAA TATTCGAGAC ATTGACAAGA TTTATGTTCG AACAGGTATC 1080
TACCATGGAG GAGAACCCTT ATGTGACAAT GTGAACACTC AAAGAGTACC TTGTTCCAAT 1140
CCCAGGTGGA ATGAATGGCT GAATTATGAT ATATACATTC CTGATCTTCC TCGTGCTGCT 1200
CGACTTTGCC TTTCCATTTG CTCTGTTAAA GGCCGAAAGG GTGCTAAAGA GGAACACTGT 1260
CCATTGGCAT GGGGAAATAT AAACTTGTTT GATTACACAG ACACTCTAGT ATCTGGAAAA 1320
ATGGCTTTGA ATCTTTGGCC AGTACCTCAT GGATTAGAAG ATTTGCTGAA CCCTATTGGT 1380
GTTACTGGAT CAAATCCAAA TAAAGAAACT CCATGCTTAG AGTTGGAGTT TGACTGGTTC 1440
AGCAGTGTGG TAAAGTTCCC AGATATGTCA GTGATTGAAG AGCATGCCAA TTGGTCTGTA 1500
TCCCGAGAAG CAGGATTTAG CTATTCCCAC GCAGGACTGA GTAACAGACT AGCTAGAGAC 1560
AATGAATTAA GGGAAAATGA CAAAGAACAG CTCAAAGCAA TTTCTACACG AGATCCTCTC 1620
TCTGAAATCA CTGAGCAGGA GAAAGATTTT CTATGGAGTC ACAGACACTA TTGTGTAACT 1680
ATCCCCGAAA TTCTACCCAA ATTGCTTCTG TCTGTTAAAT GGAATTCTAG AGATGAAGTA 1740
GCCCAGATGT ATTGCTTGGT AAAAGATTGG CCTCCAATCA AACCTGAACA GGCTATGGAA 1800
CTTCTGGACT GTAATTACCC AGATCCTATG GTTCGAGGTT TTGCTGTTCG GTGCTTGGAA 1860
AAATATTTAA CAGATGACAA ACTTTCTCAG TATTTAATTC AGCTAGTACA GGTCCTAAAA 1920
TATGAACAAT ATTTGGATAA CTTGCTTGTG AGATTTTTAC TGAAGAAAGC ATTGACTAAT 1980
CAAAGGATTG GGCACTTTTT CTTTTGGCAT TTAAAATCTG AGATGCACAA TAAAACAGTT 2040

AGCCAGAGGT TTGGCCTGCT TTTGGAGTCC TATTGTCGTG CATGTGGGAT GTATTTGAAG 2100

CACCTGAATA GGCAAGTCGA GGCAATGGAA AAGCTCATTA ACTTAACTGA CATTCTCAAA 2160

CAGGAGAGGA AGGATGAAAC ACAAAAGGTA CAGATGAAGT TTTTAGTTGA GCAAATGAGG 2220

CGACCAGATT TCATGGATGC CCTACAGGGC TTGCTGTCTC CTCTAAACCC TGCTCATCAA 2280

CTAGGAAACC TCAGGCTTAA AGAGTGTCGA ATTATGTCTT CTGCAAAAAG GCCACTGTGG 2340

TTGAATTGGG AGAACCCAGA CATCATGTCA GAGTTACTGT TTCAGAACAA TGAGATCATC 2400

TTTAAAAATG GGGATGATTT ACGGCAAGAT ATGCTAACAC TTCAAATTAT TCGTATTATG 2460

GAAAATATCT GGCAAAATCA AGGTCTTGAT CTTCGAATGT TACCTTATGG TTGTCTGTCA 2520

ATCGGTGACT GTGTGGGACT TATTGAGGTG GTGCGAAATT CTCACACTAT TATGCAAATT 2580

CAGTGCAAAG GCGGCTTGAA AGGTGCACTG CAGTTCAACA GCCACACACT ACATCAGTGG 2640

CTCAAAGACA AGAACAAAGG AGAAATATAT GATGCAGCCA TTGACCTGTT TACACGTTCA 2700

TGTGCTGGAT ACTGTGTAGC TACCTTCATT TTGGGAATTG GAGATCGTCA CAATAGTAAC 2760

ATCATGGTGA AAGACGATGG ACAACTGTTT CATATAGATT TTGGACACTT TTTGGATCAC 2820

AAGAAGAAAA AATTTGGTTA TAAACGAGAA CGTGTGCCAT TTGTTTTGAC ACAGGATTTC 2880

TTAATAGTGA TTAGTAAAGG AGCCCAAGAA TGCACAAAGA CAAGAGAATT TGAGAGGTTT 2940

CAGGAGATGT GTTACAAGGC TTATCTAGCT ATTCGACAGC ATGCCAATCT CTTCATAAAT 3000

CTTTTCTCAA TGATGCTTGG CTCTGGAATG CCAGAACTAC AATCTTTTGA TGACATTGCA 3060

TACATTCGAA AGACCCTAGC CTTAGATAAA ACTGAGCAAG AGGCTTTGGA GTATTTCATG 3120

AAACAAATGA ATGATGCACA TCATGGTGGC TGGACAACAA AAATGGATTG GATCTTCCAC 3180

ACAATTAAAC AGCATGCATT GAACTGAAAG ATAACTGAGA AAATGAAAGC TCACTCTGGA 3240

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3207 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

ATGCCTCCAA GACCATCATC AGGTGAACTG TGGGGCATCC ACTTGATGCC CCCAAGAATC 60

CTAGTAGAAT GTTTACTACC AAATGGGATG ATAGTGACTT TAGAATGCCT CCGTGAGGCT 120

ACGTTAATAA CGATAAAGCA TGAACTATTT AAAGAAGCAA GAAAATACCC TCTCCATCAA 180

CTTCTTCAAG ATGAATCTTC TTACATTTTC GTAAGTGTTA CCCAAGAAGC AGAAAGGGAA 240

GAATTTTTTG ATGAAACAAG ACGACTTTGT GACCTTCGGC TTTTTCAACC CTTTTTAAAA 300

GTAATTGAAC CAGTAGGCAA CCGTGAAGAA AAGATCCTCA ATCGAGAAAT TGGTTTTGCT 360

ATCGGCATGC CAGTGTGTGA ATTCGATATG GTTAAAGATC CAGAAGTACA GGACTTCCGA 420

AGAAATATTC TCAATGTTTG TAAAGAAGCT GTGGATCTTA GGGATCTTAA TTCACCTCAT 480

AGTAGAGCAA TGTATGTTTA TCCTCCAAAT GTAGAATCTT CACCAGAACT GCCAAAGCAC 540

ATATATAATA AATTGGATAA AGGGCAAATA ATAGTGGTGA TTTGGGTAAT AGTTTCTCCA 600

AATAATGACA AACAGAAGTA TACTCTGAAA ATCAACCATG ACTGTGTGCC AGAACAAGTA 660

ATTGCTGAAG CAATCAGGAA AAAAACTCGA AGTATGTTGC TATCATCTGA ACAACTAAAA 720

CTCTGTGTTT TAGAATATCA GGGCAAGTAT ATTTTAAAAG TGTGTGGATG TGATGAATAC 780

TTCCTAGAAA AATATCCTCT GAGTCAGTAT AAGTATATAA GAAGCTGTAT AATGCTTGGG 840

AGGATGCCCA ATTTGATGCT GATGGCTAAA GAAAGCCTCT ATTCTCAACT GCCAATGGAC 900

TGTTTTACAA TGCCATCATA TTCCAGACGC ATCTCCACAG CTACGCCATA TATGAATGGA 960

GAAACATCTA CAAAATCCCT TTGGGTTATA AATAGTGCAC TCAGAATAAA AATTCTTTGT 1020

GCAACCTATG TGAATGTAAA TATTCGAGAC ATTGACAAGA TTTATGTTCG AACAGGTATC 1080

TACCATGGAG GAGAACCCTT ATGTGATAAT GTGAACACTC AAAGAGTACC TTGTTCCAAT 1140

CCCAGGTGGA ATGAATGGCT GAATTACGAT ATATACATTC CTGATCTTCC TCGTGCTGCT 1200

CGACTTTGCC TTTCCATTTG TTCTGTTAAA GGCCGAAAGG GTGCTAAAGA GGAACACTGT 1260

CCATTGGCCT GGGGAAATAT AAACTTGTTT GATTACACAG ATACTCTAGT ATCTGGAAAA 1320

ATGGCTTTGA ATCTTTGGCC AGTACCTCAT GGACTAGAAG ATTTGCTGAA CCCTATTGGT 1380

GTTACTGGAT CAAATCCAAA TAAAGAAACT CCATGTTTAG AGTTGGAGTT TGACTGGTTC 1440

AGCAGTGTGG TAAAGTTTCC AGATATGTCA GTGATTGAAG AGCATGCCAA TTGGTCTGTA 1500

TCCCGTGAAG CAGGATTTAG TTATTCCCAT GCAGGACTGA GTAACAGACT AGCTAGAGAC 1560

AATGAATTAA GAGAAAATGA TAAAGAACAG CTCCGAGCAA TTTGTACACG AGATCCTCTA 1620

TCTGAAATCA CTGAGCAAGA GAAAGATTTT CTGTGGAGCC ACAGACACTA TTGTGTAACT 1680

ATCCCCGAAA TTCTACCCAA ATTGCTTCTG TCTGTTAAAT GGAACTCTAG AGATGAAGTA 1740

GCTCAGATGT ACTGCTTGGT AAAAGATTGG CCTCCAATCA AGCCTGAACA GGCTATGGAG 1800

CTTCTGGACT GCAATTACCC AGATCCTATG GTTCGAGGTT TTGCTGTTCG GTGCTTAGAA 1860

AAATATTTAA CAGATGACAA ACTTTCTCAG TACCTAATTC AGCTAGTACA GGTACTAAAA 1920

TATGAACAGT ATTTGGATAA CCTGCTTGTG AGATTTTTAC TCAAAAAAGC GTTAACTAAT 1980

CAAAGGATCG GTCACTTTTT CTTTTGGCAT TTAAAATCTG AGATGCACAA TAAAACAGTT 2040

AGTCAGAGGT TTGGCCTGCT TTTGGAGTCC TATTGCCGTG CATGTGGGAT GTATCTGAAG 2100

CACCTTAATA GGCAAGTTGA GGCTATGGAA AAGCTCATTA ACTTGACTGA CATTCTCAAA 2160

CAAGAGAAGA AGGATGAAAC ACAAAAGGTA CAGATGAAGT TTTTAGTTGA GCAAATGCGG 2220

CGACCAGATT TCATGGATGC TCTCCAGGGC TTTCTGTCTC CTCTAAACCC TGCTCATCAG 2280

CTGGGAAATC TCAGGCTTGA AGAGTGTCGA ATTATGTCTT CTGCAAAAAG GCCACTGTGG 2340

TTGAATTGGG AGAACCCAGA CATCATGTCA GAATTACTCT TTCAGAACAA TGAGATCATC 2400

TTTAAAAATG GGGATGATTT ACGGCAAGAT ATGCTAACCC TTCAGATTAT TCGCATTATG 2460

GAAAATATCT GGCAAAATCA AGGTCTTGAT CTTCGAATGT TACCTTATGG ATGTCTGTCA 2520

ATCGGTGACT GTGTGGGACT TATCGAGGTG GTGAGAAATT CTCACACTAT AATGCAGATT 2580

CAGTGTAAAG GAGGCCTGAA AGGTGCACTG CAGTTTAACA GCCACACACT CCATCAGTGG 2640

CTCAAAGACA AGAACAAGGG GGAAATATAT GATGCGGCCA TCGATTTGTT TACACGATCA 2700

TGTGCTGGAT ATTGTGTTGC CACCTTCATT TTGGGAATTG GAGATCGTCA CAATAGTAAT 2760

ATCATGGTTA AAGATGATGG ACAACTGTTT CATATAGATT TTGGACACTT TTTGGATCAC 2820

AAGAAGAAAA AATTTGGTTA TAAACGAGAG CGCGTGCCGT TTGTTTTGAC ACAAGATTTC 2880

TTAATAGTGA TTAGTAAAGG AGCCCAAGAA TGCACAAAGA CAAGAGAATT TGAGAGGTTT 2940

CAGGAGATGT GTTACAAGGC TTATCTAGCT ATTCGGCAGC ATGCCAATCT CTTCATAAAT 3000

CTTTTCTCAA TGATGCTTGG CTCTGGAATG CCAGAACTGC AATCTTTTGA TGATATTGCA 3060

TACATTCGAA AGACCCTAGC TTTAGATAAA ACTGAGCAAG AGGCTTTGGA GTATTTCATG 3120

AAACAAATGA ATGATGCACA CCATGGTGGC TGGACAACAA AAATGGATTG GATCTTCCAC 3180

ACAATTAAGC AGCATGCTTT GAACTGA 3207

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1080 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Met Pro Pro Arg Pro Ser Ser Gly Glu Leu Trp Gly Ile His Leu Met
1 5 10 15

Pro Pro Arg Ile Leu Val Glu Cys Leu Leu Pro Asn Gly Met Ile Val
20 25 30

Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Val Thr Ile Lys His Glu
35 40 45

Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu Leu Gln Asp
50 55 60

Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala Glu Arg Glu
65 70 75 80

Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg Leu Phe Gln
85 90 95

Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu Glu Lys Ile
100 105 110

Leu Asn Arg Glu Ile Gly Phe Ala Ile Gly Met Pro Val Cys Glu Phe
115 120 125

Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg Asn Ile Leu
130 135 140

Asn Val Cys Lys Glu Ala Val Asp Leu Arg Asp Leu Asn Ser Pro His
145 150 155 160

Ser Arg Ala Met Tyr Val Tyr Pro Pro His Val Glu Ser Ser Pro Glu
165 170 175

Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Arg Gly Gln Ile Ile Val
180 185 190

Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln Lys Tyr Thr
195 200 205

Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile Ala Glu Ala
210 215 220

Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu Gln Leu Lys
225   230   235   240

Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys Val Cys Gly
                245   250   255

Cys Asp Glu Tyr Phe Leu Glu Lys Tyr Pro Leu Ser Gln Tyr Lys Tyr
            260   265   270

Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu Lys Met Met
        275   280   285

Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys Phe Thr Met
    290   295   300

Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr Met Asn Gly
305   310   315   320

Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Arg Ala Leu Arg Ile
                325   330   335

Lys Ile Leu Cys Ala Thr Tyr Val Asn Leu Asn Ile Arg Asp Ile Asp
            340   345   350

Lys Ile Tyr Val Arg Thr Gly Ile Tyr His Gly Gly Glu Pro Leu Cys
        355   360   365

Asp Asn Val Asn Thr Gln Arg Val Pro Cys Ser Asn Pro Arg Trp Asn
    370   375   380

Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro Arg Ala Ala
385   390   395   400

Arg Leu Cys Leu Ser Ile Cys Ser Val Lys Gly Arg Lys Gly Ala Lys
                405   410   415

Glu Glu His Cys Pro Leu Ala Trp Gly Asn Ile Asn Leu Phe Asp Tyr
            420   425   430

Thr Asp Thr Leu Val Ser Gly Lys Met Ala Leu Asn Leu Trp Pro Val
        435   440   445

Pro His Gly Leu Glu Asp Leu Leu Asn Pro Ile Gly Val Thr Gly Ser
    450   455   460

Asn Pro Asn Lys Glu Thr Pro Cys Leu Glu Leu Glu Phe Asp Trp Phe
465 470 475 480

Ser Ser Val Val Lys Phe Pro Asp Met Ser Val Ile Glu Glu His Ala
485 490 495

Asn Trp Ser Val Ser Arg Glu Ala Gly Phe Ser Tyr Ser His Ala Gly
500 505 510

Leu Ser Asn Arg Leu Ala Arg Asp Asn Glu Leu Arg Glu Asn Asp Lys
515 520 525

Glu Gln Leu Lys Ala Ile Ser Thr Arg Asp Pro Leu Ser Glu Ile Thr
530 535 540

Glu Gln Glu Lys Asp Phe Leu Trp Ser His Arg His Tyr Cys Val Thr
545 550 555 560

Ile Pro Glu Ile Leu Pro Lys Leu Leu Leu Ser Val Lys Trp Asn Ser
565 570 575

Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp Trp Pro Pro
580 585 590

Ile Lys Pro Glu Gln Ala Met Glu Leu Leu Asp Cys Asn Tyr Pro Asp
595 600 605

Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys Tyr Leu Thr
610 615 620

Asp Asp Lys Leu Ser Gln Tyr Leu Ile Gln Leu Val Gln Val Leu Lys
625 630 635 640

Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu Leu Lys Lys
645 650 655

Ala Leu Thr Asn Gln Arg Ile Gly His Phe Phe Phe Trp His Leu Lys
660 665 670

Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly Leu Leu Leu
675 680 685

Glu Ser Tyr Cys Arg Ala Cys Gly Met Tyr Leu Lys His Leu Asn Arg
    690   695   700

Gln Val Glu Ala Met Glu Lys Leu Ile Asn Leu Thr Asp Ile Leu Lys
705   710   715   720

Gln Glu Arg Lys Asp Glu Thr Gln Lys Val Gln Met Lys Phe Leu Val
                725   730   735

Glu Gln Met Arg Arg Pro Asp Phe Met Asp Ala Leu Gln Gly Leu Leu
            740   745   750

Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg Leu Lys Glu
        755   760   765

Cys Arg Ile Met Ser Ser Ala Lys Arg Pro Leu Trp Leu Asn Trp Glu
    770   775   780

Asn Pro Asp Ile Met Ser Glu Leu Leu Phe Gln Asn Asn Glu Ile Ile
785   790   795   800

Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Ile
                805   810   815

Ile Arg Ile Met Glu Asn Ile Trp Gln Asn Gln Gly Leu Asp Leu Arg
            820   825   830

Met Leu Pro Tyr Gly Cys Leu Ser Ile Gly Asp Cys Val Gly Leu Ile
        835   840   845

Glu Val Val Arg Asn Ser His Thr Ile Met Gln Ile Gln Cys Lys Gly
    850   855   860

Gly Leu Lys Gly Ala Leu Gln Phe Asn Ser His Thr Leu His Gln Trp
865   870   875   880

Leu Lys Asp Lys Asn Lys Gly Glu Ile Tyr Asp Ala Ala Ile Asp Leu
                885   890   895

Phe Thr Arg Ser Cys Ala Gly Tyr Cys Val Ala Thr Phe Ile Leu Gly
            900   905   910

Ile Gly Asp Arg His Asn Ser Asn Ile Met Val Lys Asp Asp Gly Gln
        915   920   925

Leu Phe His Ile Asp Phe Gly His Phe Leu Asp His Lys Lys Lys Lys
    930   935   940

Phe Gly Tyr Lys Arg Glu Arg Val Pro Phe Val Leu Thr Gln Asp Phe
945   950   955   960

Leu Ile Val Ile Ser Lys Gly Ala Gln Glu Cys Thr Lys Thr Arg Glu
                965   970   975

Phe Glu Arg Phe Gln Glu Met Cys Tyr Lys Ala Tyr Leu Ala Ile Arg
            980   985   990

Gln His Ala Asn Leu Phe Ile Asn Leu Phe Ser Met Met Leu Gly Ser
        995   1000   1005

Gly Met Pro Glu Leu Gln Ser Phe Asp Asp Ile Ala Tyr Ile Arg Lys
    1010   1015   1020

Thr Leu Ala Leu Asp Lys Thr Glu Gln Glu Ala Leu Glu Tyr Phe Met
1025   1030   1035   1040

Lys Gln Met Asn Asp Ala His His Gly Gly Trp Thr Thr Lys Met Asp
                1045   1050   1055

Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn Xaa Lys Ile Thr
            1060   1065   1070

Glu Lys Met Lys Ala His Ser Gly
        1075   1080

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1069 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Met Pro Pro Arg Pro Ser Ser Gly Glu Leu Trp Gly Ile His Leu Met
1   5   10   15

Pro Pro Arg Ile Leu Val Glu Cys Leu Leu Pro Asn Gly Met Ile Val
            20   25   30

Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Ile Thr Ile Lys His Glu
35 40 45

Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu Leu Gln Asp
50 55 60

Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala Glu Arg Glu
65 70 75 80

Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg Leu Phe Gln
85 90 95

Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu Glu Lys Ile
100 105 110

Leu Asn Arg Glu Ile Gly Phe Ala Ile Gly Met Pro Val Cys Glu Phe
115 120 125

Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg Asn Ile Leu
130 135 140

Asn Val Cys Lys Glu Ala Val Asp Leu Arg Asp Leu Asn Ser Pro His
145 150 155 160

Ser Arg Ala Met Tyr Val Tyr Pro Pro Asn Val Glu Ser Ser Pro Glu
165 170 175

Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Lys Gly Gln Ile Ile Val
180 185 190

Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln Lys Tyr Thr
195 200 205

Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile Ala Glu Ala
210 215 220

Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu Gln Leu Lys
225 230 235 240

Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys Val Cys Gly
245 250 255

Cys Asp Glu Tyr Phe Leu Glu Lys Tyr Pro Leu Ser Gln Tyr Lys Tyr
260 265 270

Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu Met Leu Met
275 280 285

Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys Phe Thr Met
290 295 300

Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr Met Asn Gly
305 310 315 320

Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Ser Ala Leu Arg Ile
325 330 335

Lys Ile Leu Cys Ala Thr Tyr Val Asn Val Asn Ile Arg Asp Ile Asp
340 345 350

Lys Ile Tyr Val Arg Thr Gly Ile Tyr His Gly Gly Glu Pro Leu Cys
355 360 365

Asp Asn Val Asn Thr Gln Arg Val Pro Cys Ser Asn Pro Arg Trp Asn
370 375 380

Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro Arg Ala Ala
385 390 395 400

Arg Leu Cys Leu Ser Ile Cys Ser Val Lys Gly Arg Lys Gly Ala Lys
405 410 415

Glu Glu His Cys Pro Leu Ala Trp Gly Asn Ile Asn Leu Phe Asp Tyr
420 425 430

Thr Asp Thr Leu Val Ser Gly Lys Met Ala Leu Asn Leu Trp Pro Val
435 440 445

Pro His Gly Leu Glu Asp Leu Leu Asn Pro Ile Gly Val Thr Gly Ser
450 455 460

Asn Pro Asn Lys Glu Thr Pro Cys Leu Glu Leu Glu Phe Asp Trp Phe
465 470 475 480

Ser Ser Val Val Lys Phe Pro Asp Met Ser Val Ile Glu Glu His Ala
485 490 495

Asn Trp Ser Val Ser Arg Glu Ala Gly Phe Ser Tyr Ser His Ala Gly
500 505 510

Leu Ser Asn Arg Leu Ala Arg Asp Asn Glu Leu Arg Glu Asn Asp Lys
515 520 525

Glu Gln Leu Arg Ala Ile Cys Thr Arg Asp Pro Leu Ser Glu Ile Thr
530 535 540

Glu Gln Glu Lys Asp Phe Leu Trp Ser His Arg His Tyr Cys Val Thr
545 550 555 560

Ile Pro Glu Ile Leu Pro Lys Leu Leu Leu Ser Val Lys Trp Asn Ser
565 570 575

Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp Trp Pro Pro
580 585 590

Ile Lys Pro Glu Gln Ala Met Glu Leu Leu Asp Cys Asn Tyr Pro Asp
595 600 605

Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys Tyr Leu Thr
610 615 620

Asp Asp Lys Leu Ser Gln Tyr Leu Ile Gln Leu Val Gln Val Leu Lys
625 630 635 640

Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu Leu Lys Lys
645 650 655

Ala Leu Thr Asn Gln Arg Ile Gly His Phe Phe Phe Trp His Leu Lys
660 665 670

Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly Leu Leu Leu
675 680 685

Glu Ser Tyr Cys Arg Ala Cys Gly Met Tyr Leu Lys His Leu Asn Arg
690 695 700

Gln Val Glu Ala Met Glu Lys Leu Ile Asn Leu Thr Asp Ile Leu Lys
705 710 715 720

Gln Glu Lys Lys Asp Glu Thr Gln Lys Val Gln Met Lys Phe Leu Val
725 730 735

Glu Gln Met Arg Arg Pro Asp Phe Met Asp Ala Leu Gln Gly Phe Leu
740 745 750

Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg Leu Glu Glu
755 760 765

Cys Arg Ile Met Ser Ser Ala Lys Arg Pro Leu Trp Leu Asn Trp Glu
770 775 780

Asn Pro Asp Ile Met Ser Glu Leu Leu Phe Gln Asn Asn Glu Ile Ile
785 790 795 800

Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Ile
805 810 815

Ile Arg Ile Met Glu Asn Ile Trp Gln Asn Gln Gly Leu Asp Leu Arg
820 825 830

Met Leu Pro Tyr Gly Cys Leu Ser Ile Gly Asp Cys Val Gly Leu Ile
835 840 845

Glu Val Val Arg Asn Ser His Thr Ile Met Gln Ile Gln Cys Lys Gly
850 855 860

Gly Leu Lys Gly Ala Leu Gln Phe Asn Ser His Thr Leu His Gln Trp
865 870 875 880

Leu Lys Asp Lys Asn Lys Gly Glu Ile Tyr Asp Ala Ala Ile Asp Leu
885 890 895

Phe Thr Arg Ser Cys Ala Gly Tyr Cys Val Ala Thr Phe Ile Leu Gly
900 905 910

Ile Gly Asp Arg His Asn Ser Asn Ile Met Val Lys Asp Asp Gly Gln
915 920 925

Leu Phe His Ile Asp Phe Gly His Phe Leu Asp His Lys Lys Lys Lys
930 935 940

Phe Gly Tyr Lys Arg Glu Arg Val Pro Phe Val Leu Thr Gln Asp Phe
945 950 955 960

Leu Ile Val Ile Ser Lys Gly Ala Gln Glu Cys Thr Lys Thr Arg Glu
965 970 975

Phe Glu Arg Phe Gln Glu Met Cys Tyr Lys Ala Tyr Leu Ala Ile Arg
980 985 990

Gln His Ala Asn Leu Phe Ile Asn Leu Phe Ser Met Met Leu Gly Ser
995 1000 1005

Gly Met Pro Glu Leu Gln Ser Phe Asp Asp Ile Ala Tyr Ile Arg Lys
1010 1015 1020

Thr Leu Ala Leu Asp Lys Thr Glu Gln Glu Ala Leu Glu Tyr Phe Met
1025 1030 1035 1040

Lys Gln Met Asn Asp Ala His His Gly Gly Trp Thr Thr Lys Met Asp
1045 1050 1055

Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn Xaa
1060 1065

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 381 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..381

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GGA GAC GAC TTG CGA CAG GAT CAA CTT ATT CTT CAA ATC ATT TCA CTC 48
Gly Asp Asp Leu Arg Gln Asp Gln Leu Ile Leu Gln Ile Ile Ser Leu
1 5 10 15

ATG GAC AAG CTG TTA CGG AAA GAA AAT CTG GAC TTG AAA TTG ACA CCT 96
Met Asp Lys Leu Leu Arg Lys Glu Asn Leu Asp Leu Lys Leu Thr Pro
20 25 30

TAT AAG GTG TTA GCC ACC AGT ACA AAA CAT GGC TTC ATG CAG TTT ATC 144
Tyr Lys Val Leu Ala Thr Ser Thr Lys His Gly Phe Met Gln Phe Ile
35 40 45

CAG TCA GTT CCT GTG GCT GAA GTT CTT GAT ACA GAG GGA AGC ATT CAG 192
Gln Ser Val Pro Val Ala Glu Val Leu Asp Thr Glu Gly Ser Ile Gln
50 55 60

AAC TTT TTT AGA AAA TAT GCA CCA AGT GAG AAT GGG CCA AAT GGG ATT 240
Asn Phe Phe Arg Lys Tyr Ala Pro Ser Glu Asn Gly Pro Asn Gly Ile
65 70 75 80

AGT GCT GAG GTC ATG GAC ACT TAC GTT AAA AGC TGT GCT GGA TAT TGC 288
Ser Ala Glu Val Met Asp Thr Tyr Val Lys Ser Cys Ala Gly Tyr Cys
85 90 95

GTG ATC ACC TAT ATA CTT GGA GTT GGA GAC AGG CAC CTG GAT AAC CTT 336
Val Ile Thr Tyr Ile Leu Gly Val Gly Asp Arg His Leu Asp Asn Leu
100 105 110

TTG CTA ACC AAA ACA GGC AAA CTC TTC CAC ATC GAT TTC GGC CAC 381
Leu Leu Thr Lys Thr Gly Lys Leu Phe His Ile Asp Phe Gly His
115 120 125

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 127 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Gly Asp Asp Leu Arg Gln Asp Gln Leu Ile Leu Gln Ile Ile Ser Leu
1 5 10 15

Met Asp Lys Leu Leu Arg Lys Glu Asn Leu Asp Leu Lys Leu Thr Pro
20 25 30

Tyr Lys Val Leu Ala Thr Ser Thr Lys His Gly Phe Met Gln Phe Ile
35 40 45

Gln Ser Val Pro Val Ala Glu Val Leu Asp Thr Glu Gly Ser Ile Gln
50 55 60

Asn Phe Phe Arg Lys Tyr Ala Pro Ser Glu Asn Gly Pro Asn Gly Ile
65 70 75 80

Ser Ala Glu Val Met Asp Thr Tyr Val Lys Ser Cys Ala Gly Tyr Cys
85 90 95

Val Ile Thr Tyr Ile Leu Gly Val Gly Asp Arg His Leu Asp Asn Leu
100 105 110

Leu Leu Thr Lys Thr Gly Lys Leu Phe His Ile Asp Phe Gly His
115 120 125

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 393 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..393

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GGG GAT GAC TTA CGG CAG GAC ATG CTA ACG CTG CAG ATG ATT CGC ATC 48
Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Met Ile Arg Ile
1 5 10 15

ATG AGC AAG ATC TGG GTC CAG GAG GGG CTG GAC ATG CGC ATG GTC ATC 96
Met Ser Lys Ile Trp Val Gln Glu Gly Leu Asp Met Arg Met Val Ile
20 25 30

TTC CGC TGC TTC TCC ACC GGC CGG GGC AGA GGG ATG GTG GAG ATG ATC 144
Phe Arg Cys Phe Ser Thr Gly Arg Gly Arg Gly Met Val Glu Met Ile
35 40 45

CCT AAT GCT GAG ACC CTG CGT AAG ATC CAG GTG GAG CAT GGG GTG ACC 192
Pro Asn Ala Glu Thr Leu Arg Lys Ile Gln Val Glu His Gly Val Thr
50 55 60

GGC TCG TTC AAG GAC CGG CCC CTG GCA GAC CGG CTG CAG AAA CAC AAC 240
Gly Ser Phe Lys Asp Arg Pro Leu Ala Asp Arg Leu Gln Lys His Asn
65 70 75 80

CCT GGG GAG GAC GAG TAT GAG AAG GCT GTG GAG AAC TTT ATC TAC TCC 288
Pro Gly Glu Asp Glu Tyr Glu Lys Ala Val Glu Asn Phe Ile Tyr Ser
85 90 95

TGC GCT GGC TGC TGC GTG GCC ACG TAC GTC TTG GGC ATC TGT GAC CGA 336
Cys Ala Gly Cys Cys Val Ala Thr Tyr Val Leu Gly Ile Cys Asp Arg
100 105 110

CAT AAT GAC AAC ATC ATG CTG AAG ACC ACT GGT CAC ATG TTC CAC ATC 384
His Asn Asp Asn Ile Met Leu Lys Thr Thr Gly His Met Phe His Ile
115 120 125

GAC TTC GGC 393
Asp Phe Gly
130

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 131 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Met Ile Arg Ile
1 5 10 15

Met Ser Lys Ile Trp Val Gln Glu Gly Leu Asp Met Arg Met Val Ile
20 25 30

Phe Arg Cys Phe Ser Thr Gly Arg Gly Arg Gly Met Val Glu Met Ile
35 40 45

Pro Asn Ala Glu Thr Leu Arg Lys Ile Gln Val Glu His Gly Val Thr
50 55 60

Gly Ser Phe Lys Asp Arg Pro Leu Ala Asp Arg Leu Gln Lys His Asn
65 70 75 80

Pro Gly Glu Asp Glu Tyr Glu Lys Ala Val Glu Asn Phe Ile Tyr Ser
85 90 95

Cys Ala Gly Cys Cys Val Ala Thr Tyr Val Leu Gly Ile Cys Asp Arg
100 105 110

His Asn Asp Asn Ile Met Leu Lys Thr Thr Gly His Met Phe His Ile
115 120 125

Asp Phe Gly
130

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```
Gly Asp Asp Leu Arg Gln Asp Gln Leu Val Val Gln Ile Ile Ser Leu
1               5                   10                  15

Met Asn Glu Leu Leu Lys Asn Glu Asn Val Asp Leu Lys Leu Thr Pro
            20                  25                  30

Tyr Lys Ile Leu Ala Thr Gly Pro Gln Glu Gly Ala Ile Glu Phe Ile
        35                  40                  45

Pro Asn Asp Thr Leu Ala Ser Ile Leu Ser Lys Tyr His Gly Ile Leu
    50                  55                  60

Gly Tyr
65
```

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```
Leu Lys Leu His Tyr Pro Asp Glu Asn Ala Thr Leu Gly Val Gln Gly
1               5                   10                  15

Trp Val Leu Asp Asn Phe Val Lys Ser Cys Ala Gly Tyr Cys Val Ile
            20                  25                  30

Thr Tyr Ile Leu Gly Val Gly Asp Arg His Leu Asp Asn Leu Leu Val
        35                  40                  45

Thr Pro Asp Gly His Phe Phe His Ala Asp Phe Gly
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

```
Gly Asp Asp Leu Arg Gln Asp Gln Leu Ile Leu Gln Ile Ile Ser Leu
1               5                   10                  15

Met Asp Lys Leu Leu Arg Lys Glu Asn Leu Asp Leu Lys Leu Thr Pro
            20                  25                  30

Tyr Lys Val Leu Ala Thr Ser Thr Lys His Gly Phe Met Gln Phe Ile
        35                  40                  45

Gln Ser Val Pro Val Ala Glu Val Leu Asp Thr Glu Gly Ser Ile Gln
    50                  55                  60

Asn Phe
65
```

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```
Phe Arg Lys Tyr Ala Pro Ser Glu Asn Gly Pro Asn Gly Ile Ser Ala
1               5                   10                  15

Glu Val Met Asp Thr Tyr Val Lys Ser Cys Ala Gly Tyr Cys Val Ile
            20                  25                  30

Thr Tyr Ile Leu Gly Val Gly Asp Arg His Leu Asp Asn Leu Leu Leu
        35                  40                  45

Thr Lys Thr Gly Lys Leu Phe His Ile Asp Phe Gly
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

```
Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Ile Ile Arg Ile
1               5                   10                  15

Met Glu Asn Ile Trp Gln Asn Gln Gly Leu Asp Leu Arg Met Leu Pro
            20                  25                  30

Tyr Gly Cys Leu Ser Ile Gly Asp Cys Val Gly Leu Ile Glu Val Val
        35                  40                  45

Arg Asn Ser His Thr Ile Met Gln Ile Gln Cys Lys Gly Gly Leu Lys
    50                  55                  60

Gly Ala Leu Gln Phe Asn Ser His Thr Leu His Gln Trp Leu Lys Asp
65                  70                  75                  80

Lys Asn Lys Gly Glu
                85
```

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
Ile Tyr Asp Ala Ala Ile Asp Leu Phe Thr Arg Ser Cys Ala Gly Tyr
1               5                   10                  15

Cys Val Ala Thr Phe Ile Leu Gly Ile Gly Asp Arg His Asn Ser Asn
            20                  25                  30

Ile Met Val Lys Asp Asp Gly Gln Leu Phe His Ile Asp Phe Gly
35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

```
Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Met Ile Arg Ile
1               5                   10                  15

Met Ser Lys Ile Trp Val Gln Glu Gly Leu Asp Met Arg Met Val Ile
            20                  25                  30

Phe Arg Cys Phe Ser Thr Gly Arg Gly Arg Gly Met Val Glu Met Ile
        35                  40                  45

Pro Asn Ala Glu Thr Leu Arg Lys Ile Gln Val Glu His Gly Val Thr
    50                  55                  60

Gly Ser
65
```

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

```
Phe Lys Asp Arg Pro Leu Ala Asp Arg Leu Gln Lys His Asn Pro Gly
1               5                   10                  15

Glu Asp Glu Tyr Glu Lys Ala Val Glu Asn Phe Ile Tyr Ser Cys Ala
            20                  25                  30

Gly Cys Cys Val Ala Thr Tyr Val Leu Gly Ile Cys Asp Arg His Asn
        35                  40                  45

Asp Asn Ile Met Leu Lys Thr Thr Gly His Met Phe His Ile Asp Phe
    50                  55                  60
```

Gly
65

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

```
Gly Asp Asp Leu Arg Gln Asp Leu Leu Gln Ile Ile Met Glu Leu Asp
1               5                   10                  15

Leu Pro Tyr Leu Thr Gly Gly Ile Glu Ile Asn Gly Ile Gly Leu Asn
            20                  25                  30

Ile Asp Phe Val Ser Cys Ala Gly Tyr Cys Val Thr Tyr Ile Leu Gly
        35                  40                  45

Gly Asp Arg His Asp Asn Gly Leu Phe His Ile Asp Phe Gly
    50                  55                  60
```

** TX STATUS REPORT ** AS OF JUN 03 1999 16:51 PAGE.01

FULBRIGHT&JAWORSKI 24

	DATE	TIME	TO/FROM		MODE	MIN/SEC	PGS	JOB#	STATUS
30	06/03	16:50	828 5427	2854	G3--S	00'37"	001	177	OK

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805 THIRD AVENUE
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June 3, 1999

Via Facsimile 011-44-171-828-5427 & Mail

Kati Hudson, Ph.D.
Ludwig Institute for Cancer Research
6th Floor
Glen House, Stag Place
London SW1E 5AG
ENGLAND

Re: SSX and SSX5 Genes
Our Ref: LUD 5480- JEL/NDH

Dear Kati:

In connection with the above referenced application, I have Alex Knuth's assignment, but no others.

My understanding is that Gure, Tsang, Scanlan & Chen assign to Sloan Kettering, while Sahin, Pfreundschuh and Old assign to LICR. Can you confirm this/ Once I have the information, I will prepare the relevant documents

Very truly yours,

Norman D. Hanson

NDH/dms
Enclosures

cc: Edward A. McDermott, Esq.
Dr. Lloyd J. Old

579716.1

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
LUD 256-JEL/CHI

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

POLYPEPTIDES HAVING KINASE ACTIVITY, THEIR PREPARATION AND USE

the specification of which (check only one item below):

☐ is attached hereto.

☒ was filed as United States application

Serial No. 08/162,081

on December 10, 1993

and was amended

on ____________ (if applicable).

☒ was filed as PCT international application

Number PCT/GB93/00761

on 13 April 1993

and was amended under PCT Article 19

on ____________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowlege the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Great Britain	9208135.5	13 April 1992	☒ YES ☐ NO
PCT	PCT/GB93/00761	13 April 1993	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

Combined Declaration For Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
LUD 256-JEL/NDH

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowlege the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS			STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE		PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S					
PCT APPLICATION NO	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			
PCT/GB93/00761	13 April 1993				

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number) John E. Lynch, Reg. No. 20,940; Peter F. Felfe, Reg. No. 20,297; Norman D. Hanson, Reg. No. 30,946; Christine H. Tsai, Reg. No. 34,266; Alfred H. Hemingway, Jr., Reg. No. 26,736; Vincent M. Fazzari, Reg. No. 26,879; Andrew L. Tiajoloff, Reg. No. 31,575; F. Brice Faller, Reg. No. 29,532.

Send Correspondence to:	Direct Telephone Calls to: (name and telephone number)
FELFE & LYNCH 805 Third Avenue New York, New York 10022	Christine H. Tsai (212) 688-9200

201	FULL NAME OF INVENTOR	FAMILY NAME: HILES	FIRST GIVEN NAME: Ian	SECOND GIVEN NAME: D.
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House St.	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtaud Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London WIP 8BT
202	FULL NAME OF INVENTOR	FAMILY NAME: FRY	FIRST GIVEN NAME: Michael	SECOND GIVEN NAME: J.
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtaud Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London WIP 8BT
203	FULL NAME OF INVENTOR	FAMILY NAME: DHAND	FIRST GIVEN NAME: Ritu	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtauld Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London W1P 8BT

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Hiles Fry Dhand

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
I Hiles	Michael J. Fry	[signature]
DATE 14/1/94	DATE 6-1-94	DATE 13/1/94

Combined Declaration For Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER

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U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.			STATUS (Check one)		
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)	PATENTED	PENDING	ABANDONED
PCT/GB93/00761	13 April 1993				

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Send Correspondence to:
FELFE & LYNCH
805 Third Avenue
New York, New York 10022

Direct Telephone Calls to: (name and telephone number)
Christine H. Tsai
(212) 688-9200

204	FULL NAME OF INVENTOR	FAMILY NAME: WATERFIELD	FIRST GIVEN NAME: Michael	SECOND GIVEN NAME: D.
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtaud Building	CITY: 91 Riding Street House	STATE & ZIP CODE/COUNTRY: London W1P 8BT
205	FULL NAME OF INVENTOR	FAMILY NAME: PARKER	FIRST GIVEN NAME: Peter	SECOND GIVEN NAME: J.
	RESIDENCE & CITIZENSHIP	CITY: Lincoln's Inn Fields	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: P.O. Box 123	CITY: Lincoln's Inn Fields	STATE & ZIP CODE/COUNTRY: London WC2A 3PX
206	FULL NAME OF INVENTOR	FAMILY NAME: OTSU	FIRST GIVEN NAME: Masayuki	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtaud Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London W1P 8BT

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Parker Otsu

SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
M. Waterfield	P. J. Parker	Masayuki Otsu
DATE 10 January '94	DATE 18 January 1994	DATE 24 January 1994

PTO 1391 (REV. 10-83) U.S. DEPARTMENT OF COMMERCE-Patent and Trademark Office

Combined Declaration For Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
LUD 256-JEI/NDH

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowlege the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS			STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE		PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			
PCT/GB93/00761	13 April 1993				

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New York, New York 10022

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Christine H. Tsai
(212) 688-9200

207	FULL NAME OF INVENTOR	FAMILY NAME: PANAYOUTOU	FIRST GIVEN NAME: George	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtauld Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London W1P 8BT
208	FULL NAME OF INVENTOR	FAMILY NAME: VOLINIA	FIRST GIVEN NAME: Stefano	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtauld Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London W1P 8BT
209	FULL NAME OF INVENTOR	FAMILY NAME: GOUT	FIRST GIVEN NAME: Ivan	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: 91 Riding House Street	STATE OR FOREIGN COUNTRY: London	COUNTRY OF CITIZENSHIP: Great Britain
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Courtauld Building	CITY: 91 Riding House Street	STATE & ZIP CODE/COUNTRY: London W1P 8BT

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 207	SIGNATURE OF INVENTOR 208	SIGNATURE OF INVENTOR 209
George Panayotou	Stefano Volinia	[illegible]
DATE 6.1.94	DATE 12.1.94	DATE 10.1.94.